Canberra Health Services | ACT Government

Our reference: **CHSFOI25-26.35**

[redacted]

Dear [redacted],

DECISION ON YOUR ACCESS APPLICATION

I refer to your application under section 30 of the *Freedom of Information Act 2016* (FOI Act), received originally by Justice and Community Safety Directorate and transferred to Canberra Health Services (the Directorate) on **Tuesday 24 February 2026**.

This application requested access to:

1. *'The name of each commercial RT-PCR test kit used and a copy of the Material/Product/Operational data sheets supplied by the respective test kit manufacturers.*
2. *If an in-house RT-PCR kit was also used, a copy of the Material/Product/Operational data sheets for it.*
3. *For each of the said RT-PCR kits, the genetic sequence of the primers for the SARS-CoV-2 contagion used by the Department of Health for the execution of the tests.*
4. *For each of the said RT-PCR kits, the number of cycles they were generically operated at.*
5. *Full citation of all peer-reviewed published scientific papers relied upon by the Department of Health for establishment of a purified isolate of the SARS CoV-2 virus or fragments thereof and genetic sequencing thereof.'*

I am an Information Officer appointed by the Chief Executive Officer of the Directorate under section 18 of the FOI Act to deal with access applications made under Part 5 of the Act. The Directorate was required to provide a decision on your access application by **Friday 10 April 2026**.

I have identified 18 documents holding the information within scope of your access application. These are outlined in the schedule of documents included at Attachment A to this decision letter.

Decisions

I have decided to:

- grant full access to 8 documents; and
- grant partial access to 10 documents.

My access decisions are detailed further in the following statement of reasons and the documents released to you are provided as Attachment B to this letter.

In reaching my access decision, I have taken the following into account:

- The FOI Act;
- The contents of the documents that fall within the scope of your request; and
- The *Human Rights Act 2004*.

Full Access
I have decided to grant full access to the documents at references: 1-8.

Partial Access
I have decided to grant partial access to the documents at references: 9-18.

Public Interest Factors Favouring Disclosure
The following factors were considered relevant in favour of the disclosure of the documents:

- Schedule 2, 2.1 (a)(i) promote open discussion of public affairs and enhance the government's accountability; and
- Schedule 2, 2.1 (a)(ii) contribute to positive and informed debate on important issues or matters of public interest.

Public Interest Factors Favouring Non-Disclosure
The following factors were considered relevant in favour of the non-disclosure of the documents:

- Schedule 2, 2.2 (a)(ii) prejudice the protection of an individual's right to privacy or any other right under the Human Rights Act 2004; and
- Schedule 2, 2.2(a)(iii) prejudice security, law enforcement or public safety.

On balance, the factors favouring disclosure did not outweigh the factor favouring non-disclosure as the redacted information contains names of constituents that are non-ACT Government employees, information that could prejudice the security of an agency. Therefore, I determined the information identified is contrary to the public interest and I have decided not to disclose this information.

Charges
Processing charges are not applicable to this request.

Disclosure Log
Under section 28 of the FOI Act, the Directorate maintains an online record of access applications called a disclosure log. The scope of your access application, my decision and documents released to you will be published in the disclosure log not less than three days but not more than 10 days after the date of this decision. Please be aware that no personal information will be included in the published information all other information will be published on https://www.act.gov.au/open/foi-disclosure-logs/act-health-directorate-and-canberra-health-services-disclosure-logs.

Ombudsman review
My decision on your access request is a reviewable decision as identified in Schedule 3 of the FOI Act. You have the right to seek Ombudsman review of this outcome under section 73 of the Act within 20 working days from the day that my decision is published on the Directorate's disclosure log, or a longer period allowed by the Ombudsman.

If you wish to request a review of my decision you may write to the Ombudsman at:

The ACT Ombudsman
GPO Box 442
CANBERRA ACT 2601
Via email: ACTFOI@ombudsman.gov.au
Website: ombudsman.act.gov.au

ACT Civil and Administrative Tribunal (ACAT) review

Under section 84 of the Act, if a decision is made under section 82(1) on an Ombudsman review, you may apply to the ACAT for review of the Ombudsman decision. Further information may be obtained from the ACAT at:

ACT Civil and Administrative Tribunal
Allara House
15 Constitution Avenue
GPO Box 370
Canberra City ACT 2601
Telephone: (02) 6207 1740
http://www.acat.act.gov.au/

Further assistance

Should you have any queries in relation to your request, please do not hesitate to contact the FOI Coordinator on (02) 5124 9831 or email HealthFOI@act.gov.au.

Yours sincerely

Greg Brylski
Executive Director
ACT Pathology
Canberra Health Services

25 March 2026

ACT Government

FREEDOM OF INFORMATION - SCHEDULE OF DOCUMENTS

FILE NUMBER:	WHAT ARE THE PARAMETERS OF THE REQUEST:	APPLICANT NAME & TYPE:
CHSFOI25-26.35	*1. The name of each commercial RT-PCR test kit used and a copy of the Material/Product/Operational data sheets supplied by the respective test kit manufacturers.* *2. If an in-house RT-PCR kit was also used, a copy of the Material/Product/Operational data sheets for it.* *3. For each of the said RT-PCR kits, the genetic sequence of the primers for the SARS-CoV-2 contagion used by the Department of Health for the execution of the tests.* *4. For each of the said RT-PCR kits, the number of cycles they were generically operated at.* *5. Full citation of all peer-reviewed published scientific papers relied upon by the Department of Health for establishment of a purified isolate of the SARS CoV-2 virus or fragments thereof and genetic sequencing thereof.'*	[redacted]

Reference No.	Page No.	Description	Document Date	Status Decision	Factor	Open Access release status
1	1	Combined PCR Table	26-February-2026	Full Release		YES
2	2 - 32	IFU3 - MP9.52 Aptima SARS-CoV-2 Assay IFU AW-21528-001_001_01	01-October-2021	Full Release		YES
3	33 - 86	IFU4 - cobas® 6800 Sars-Cov-2 Qualitative	01-January-2022	Full Release		YES
4	87 - 106	IFU5 - Xpert Xpress SARS-CoV-2 Assay ENGLISH Package Insert 302-3562 Rev. F	01-January-2021	Full Release		YES
5	107 - 171	IFU6 - MP9.53 Seegene Allplex SARS-CoV-2 user manual	01-August-2021	Full Release		YES
6	172 - 204	IFU7 - 63 Xpert Xpress CoV-2 FLU RSV plus Australia Laboratory IFU 302-8927 Rev. A	01-July-2021	Full Release		YES
7	205 - 235	IFU7 - 63 Xpert Xpress SARS-CoV-2 Flu RSV CE IVD English Package Insert 302-5159 Rev. D	01-May-2022	Full Release		YES
8	236 - 290	IFU8 - MP9.55 Seegene Allplex SARS-CoV-2_FluA_FluB_RSV user manual	01-September-2020	Full Release		YES
9	291 - 304	IFU9 - Ausdiagnostics SARS COV 2 Flu RSV (8 Well) 17.04.2020	17-April-2020	Partial Release	Schedule 2.2(a)(ii) Privacy	YES
10	305 - 328	SOP1 - 35 Coronavirus SARS-COV-2 Testing	08-July-2020	Partial Release	Schedule 2.2(a)(ii) Privacy Schedule 2.2(a)(iii) Security, law enforcement or public safety	YES
11	329 - 352	SOP2 - 35 Coronavirus SARS-COV-2 Testing	08-July-2020	Partial Release	Schedule 2.2(a)(ii) Privacy Schedule 2.2(a)(iii) Security, law enforcement or public safety	YES
12	353 - 368	SOP3 - 39 Panther Aptima SARS-COV-2	24-June-2022	Partial Release	Schedule 2.2(a)(ii) Privacy Schedule 2.2(a)(iii) Security, law enforcement or public safety	YES
13	369 - 380	SOP4 - 46. SARSCoV2 Testing by cobas® 6800	29-June-2022	Partial Release	Schedule 2.2(a)(ii) Privacy	YES
14	381 - 390	SOP5 - 37 SARS-COV-2 Testing by GeneXpert	06-July-2020	Partial Release	Schedule 2.2(a)(ii) Privacy	YES
15	391 - 408	SOP6 - 38 SeeGene Allplex SARS-COV-2 Assay	24-June-2022	Partial Release	Schedule 2.2(a)(ii) Privacy Schedule 2.2(a)(iii) Security, law enforcement or public safety	YES
16	409 - 424	SOP7 - 37 SARS-COV-2 FLU RSV Testing by GeneXpert	09-December-2022	Partial Release	Schedule 2.2(a)(ii) Privacy Schedule 2.2(a)(iii) Security, law enforcement or public safety	YES
17	425 - 438	SOP8 - 48 Seegene Allplex SARSCOV2 FLUAB RSV Assay	27-June-2022	Partial Release	Schedule 2.2(a)(ii) Privacy	YES
18	439 - 464	SOP9 - 36 Ausdiagnostics FLU RSV SARS-COV-2 Assay	08-July-2020	Partial Release	Schedule 2.2(a)(ii) Privacy	YES
Total Number of Documents						
18						

Please be aware that under the *Freedom of Information Act 2016* , some of the information provided to you will be released to the public through the ACT Government's Open Access Scheme. The Open Access release status column of the table below indicates what documents are intended for release online through open access. Personal information or business affairs information will not be made available under this policy. If you think the content of your request would contain such information, please inform the contact officer immediately. Information about what is published on open access is available online at: https://www.act.gov.au/open-access

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Serial	Test name	In house / commercial	Platform	Test kit	Company	Genetic sequence if available	Amplification cycles	Reference papers	Attached IFU	Attached SOP	Comment
1	Coronavirus (SARS-CoV-2)	In-house	Roche LightCycler 2.0	In-house	In-house	See SOP1	45	See SOP1	n/a	SOP1	
2	Coronavirus (SARS-CoV-2)	In-house	Roche LightCycler 480	In-house	In-house	See SOP2	50	See SOP2	n/a	SOP2	
3	Aptima™ SARS-CoV-2 Assay	Commercial	Panther System	Aptima™ SARS-CoV-2 Assay	Hologic	Unavailable	N/A		IFU3	SOP3	Method: Transcription mediated amplification (TMA) TMA operates at a single, constant temperature, eliminating the need for a thermal cycler. Does not operate on a fixed cycle number. It produces copies per cycle, generating a billion-fold increase in target nucleic acid.
4	cobas® SARS-CoV-2 Qualitative	Commercial	Cobas 6800	cobas® SARS-CoV-2 Qualitative	Roche	Unavailable	Not in IFU		IFU4	SOP4	Automated system decide the positivity based on an algorithm determined by the manufacturer
5	Xpert® Xpress SARS-CoV-2	Commercial	Genexpert	Xpert® Xpress SARS-CoV-2	Cepheid	Unavailable	45		IFU5	SOP5	
6	Allplex™ SARS-CoV-2 Assay	Commercial	Bio-Rad CFX-96	Allplex™ SARS-CoV-2 Assay	Integrated Science	Unavailable	45		IFU6	SOP6	
7	Xpert® Xpress CoV-2/Flu/RSV	Commercial	Genexpert	Xpert® Xpress CoV-2/Flu/RSV	Cepheid	Unavailable	45		IFU7	SOP7	
8	Allplex™ SARS-CoV-2-FluA-FluB-RSV Assay	Commercial	Bio-Rad CFX-96	Allplex™ SARS-CoV-2-FluA-FluB-RSV Assay	Integrated Science	Unavailable	42		IFU8	SOP8	
9	SARS-COV-2, INFLUENZA AND RSV 8-WELL	Commercial	HighPlex	SARS-COV-2, INFLUENZA AND RSV 8-WELL	Ausdiagnostics	Unavailable	see comment		IFU9	SOP9	Method: multiplex-tandem polymerase chain reaction, which includes initial cycling with a large number of primers, covering many different targets, are added to the sample. This mixture is subjected to a limited number of PCR cycles (typically 15 to 18) then step 2 designed to amplify a smaller section within the target sequences produced in Step1

Aptima™ SARS-CoV-2 Assay (Panther™ System)

For *in vitro* diagnostic use.

For U.S. Export only.

CONTENTS

General Information

Intended Use

The Aptima™ SARS-CoV-2 assay is a nucleic acid amplification *in vitro* diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate or nasal aspirates obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria, as well as upper respiratory specimens (such as nasopharyngeal, nasal, mid-turbinate or oropharyngeal swab specimens) collected from any individual, including from individuals without symptoms or other reasons to suspect COVID-19 infection.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory swab specimens (i.e. nasopharyngeal, nasal, mid-turbinate, or oropharyngeal swabs), where each specimen is collected under observation or by a healthcare provider using individual vials containing transport media. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing. For specific patients, whose specimen(s) were the subject of pooling, a notice that pooling was used during testing must be included when reporting the result to the healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima SARS-CoV-2 assay on the Panther™ and Panther Fusion™ system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures.

Summary and Explanation of the Test

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.[1]

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become

infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.[2] These droplets also can land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person.[3] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[4,5]

Principles of the Procedure

The Aptima SARS-CoV-2 assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands. The Aptima SARS-CoV-2 assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labeled with different acridinium ester (AE) molecules. The AE labeled probes combine with amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labeled hybrids is measured as photon signals in a luminometer, and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labeled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the "flasher" kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the "glower" kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

The Aptima SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction, using the same “glower” kinetic type. The two regions are not differentiated and amplification of either or both regions leads to RLU signal. The assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator’s Manual*.

B. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

C. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

D. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

E. If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

F. Use only supplied or specified disposable laboratory ware.

G. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).

H. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

I. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes, Hologic Specimen Lysis Tubes, the Aptima Multitest Collection Kit, the Aptima Unisex Specimen Collection Kit and the Aptima Specimen Transfer Kit pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

M. Do not use the reagents and controls after the expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 5), and *Panther System Test Procedure* (page 15) for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther system verifies reagent levels.

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

R. Testing of pooled specimens may impact the detection capability of the Aptima SARS-CoV-2 Assay and decrease sensitivity.

S. A reagent in this kit is labeled with risk and safety symbols.

Note: For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.

Selection Reagent
BORIC ACID 1-5%
WARNING
H315 - Causes skin irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2 Amplification Reagent

Aptima SARS-CoV-2 Enzyme Reagent

Aptima SARS-CoV-2 Probe Reagent

Aptima SARS-CoV-2 Internal Control

Aptima SARS-CoV-2 Positive Control

Aptima SARS-CoV-2 Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2 Amplification Reconstitution Solution

Aptima SARS-CoV-2 Enzyme Reconstitution Solution

Aptima SARS-CoV-2 Probe Reconstitution Solution

Aptima SARS-CoV-2 Selection Reagent

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2 Target Capture Reagent

Aptima Wash Solution

Aptima Buffer for Deactivation Fluid

Aptima Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Controls are stable until the date indicated on the vials.

H. Reagents stored on-board the Panther System have 72 hours of on-board stability.

I. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

J. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

K. Do not freeze the reagents.

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Aptima SARS-CoV-2 assay, this includes NP, nasal, mid-turbinate and OP swab specimens, or nasopharyngeal wash/aspirate and nasal aspirate specimen collection in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM).

Samples - Represents a more generic term to describe any material for testing on the Panther System including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube, Hologic Specimen Lysis Tube with solid cap, Aptima Specimen Transfer Tube, Aptima Multitest Transport Tube, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

Swab Specimen Collection

Collect NP swab, nasal swab, and OP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies or STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of OP and nasal swab samples.

After collection, specimens collected in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube or transfer tubes as described in the specimen processing section below. Remaining specimen volumes can be stored at ≤-70°C.

After collection, specimens in the Aptima Multitest Tube may be stored at 2°C to 30°C up to 6 days.

Note: *It is recommended that specimens transferred to the Aptima Multitest Tube are stored capped and upright in a rack.*

The following types of VTM/UTM can be used.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

Note: *Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.*

Nasopharyngeal Wash/aspirate and Nasal Aspirate Specimen Collection

Collect nasopharyngeal wash/aspirate and nasal aspirate specimens according to standard techniques.

Specimen Processing

Capped Workflow using Aptima SARS-CoV-2 Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther system, transfer 500 µL of the collected specimen* to a Panther Fusion Specimen Lysis Tube.

Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Processing using the Aptima Specimen Transfer Tube

A. Prior to testing on the Panther system, transfer 1 mL of the collected specimen* to an Aptima Specimen Transfer Tube**.

***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

****Note:** Alternatively, an unused Aptima Multitest Tube, or Aptima Unisex Tube can be used.*

B. Recap the Aptima Specimen Transfer Tube tightly.

C. Gently invert the tube 2 to 3 times to ensure complete mixture of the specimen.

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. After placing the collected specimen* into the Aptima Multitest Tube using the Aptima Multitest Collection Kit, no further processing is required.

***Note:** Allow specimen to reach room temperature prior to processing.*

Uncapped Workflow using Aptima SARS-CoV-2 Assay Software

Specimen Processing using the Panther Fusion Specimen Lysis Tube

A. Uncap the Panther Fusion Specimen Lysis Tube with penetrable cap. The penetrable cap can be retained or a replacement solid cap can be used in the next step.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Panther Fusion Specimen Lysis Tube, with penetrable cap or replacement solid cap.

C. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

D. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

***Note:** Failure to remove bubbles may affect assay processing and cause invalid results.*

E. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. Uncap the Hologic Specimen Lysis Tube with solid cap and retain the cap.

B. Prior to testing on the Panther system, transfer 500 µL of the specimen to the Hologic Specimen Lysis Tube with solid cap.

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture.

D. To avoid contact with the top of the tube, loosen the cap and place the sample tube into the sample rack.

E. Remove and discard the cap. To avoid contamination, do not pass the cap over any other sample racks or sample tubes. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube (for example, use the tip of a sterile swab or similar method).

Note: *Failure to remove bubbles may affect assay processing and cause invalid results.*

F. Place the rack retainer on the sample rack and load the rack into the instrument.

Specimen Processing for Specimen Collected with the Aptima Multitest Collection Kit

A. Obtain and follow instructions for Panther Fusion Specimen Lysis Tube (Step A), and Hologic Specimen Lysis Tube with Solid Cap (Step A).

B. Prior to testing on the Panther system, transfer 500 µL of the collected specimen from the Aptima Multitest Tube to a Panther Fusion Specimen Lysis Tube, or Hologic Specimen Lysis Tube as described in the specimen processing sections above.

Sample Storage

A. Samples on board the Panther system may be archived for additional testing at a later time.

B. Storing samples before or after testing

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition:
   - 2°C to 30°C up to 6 days
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped,
   - Capped workflows

     Remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.
   - Uncapped workflows

     Remove the solid cap and place a new solid cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

     Note: *Replacement tube closures and tube plugs should not be used to cover tubes when centrifuging, freezing, or shipping.*

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 6.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Specimen Pooling - Determining Appropriate Strategy for Implementation and Monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow.

Preparing Samples for Pooling

The following upper respiratory tract specimens may be tested with sample pooling: nasopharyngeal, oropharyngeal, mid-turbinate, and nasal swab specimens collected into VTM/UTM, saline, Liquid Amies and specimen transport media (STM). Only specimens collected into a single type of media may be combined for each sample pool. For example, specimens collected in VTM/UTM should not be combined into a pool with specimens collected in Liquid Amies. Additionally, both neat specimens (those not prepared with STM for testing) and specimens prepared with STM for testing may be included in sample pooling. Each sample pool must be comprised of only neat or only STM prepared specimens. Recommended sample pooling workflow options for different specimen types are provided below.

For Specimens Collected in VTM/UTM, Saline or Liquid Amies

Customers may choose from one of the following two options to perform specimen processing for pooled samples using Specimen Lysis Tubes with the Aptima SARS-CoV-2 assay.

Note: *Hologic testing was performed using pooled samples generated from samples collected in a single collection medium type (i.e., VTM/UTM). Combination of multiple transport media types (e.g., VTM/UTM, saline, and Liquid Amies) in a single pool has not been evaluated.*

Option 1:
Specimen Preparation Instructions for Neat Samples Pooled Directly into a Specimen Lysis Tube (Hologic SLT and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies by transferring samples directly into a Specimen Lysis Tube (Hologic SLT or Panther Fusion SLT).

A. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. The required specimen to STM ratio in the assay test sample must be maintained for sample pooling. For example, if a pool size of 5 specimens is being utilized, 100 µL of each individual specimen (500 µL total) is required.

B. Uncap the Specimen Lysis Tube and retain the cap.

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the specimen collection container to the Specimen Lysis Tube.

D. Ensure homogeneous mixing of each prepared sample pool.

Note: Retain the individual specimens for additional testing, if required.

Option 2:
Specimen Preparation Instructions for Samples Pooled Prior to Transferring to a Specimen Lysis Tube (Hologic SLT and Panther Fusion SLT)

Perform the following procedure when pooling specimens collected in VTM/UTM, saline, or Liquid Amies, by pooling the samples prior to transferring into a Specimen Lysis Tube (Hologic SLT or Panther Fusion SLT).

A. Obtain a generic empty tube. This tube will not be loaded on the Panther System for testing.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented.

   Note: *The total volume of the pooled specimens must be greater than 500* µL to support transfer into a Specimen Lysis Tube.

C. Prior to testing on the Panther system, carefully transfer *500* µL of the pooled specimens from the generic tube into a Specimen Lysis Tube (Hologic SLT or Panther Fusion SLT).

D. Ensure homogenous mixing of each prepared sample pool.

   Note: Retain the individual specimens for additional testing, if required.

Option 3:

Specimen Preparation Instructions for Specimens Previously Transferred into Specimen Lysis Tubes and Pooled into an Empty Tube

Perform the following procedure when pooling specimens from Specimen Lysis Tubes (Hologic SLT or Panther Fusion SLT) by transferring directly into an empty tube per specifications in the Panther or Panther Fusion System Operators Manual.

A. Obtain a Panther system compatible empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens previously transferred into a Specimen Lysis Tube do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Specimen Lysis Tubes (Hologic SLT or Panther Fusion SLT) to the Panther system compatible empty tube.

D. Ensure homogeneous mixing of each prepared sample pool.

E. Retain the individual specimens for additional testing if required.

For Specimens Collected in Aptima Multitest Transport Tubes

Specimen Preparation Instructions for Samples Pooled Directly into a Generic Tube

Perform the following procedure when pooling specimens collected in Aptima Multitest Transport Tubes by transferring individual specimens directly into an empty tube per specifications in the *Panther or Panther Fusion System Operators Manual*.

A. Obtain a Panther system compatible empty tube.

B. Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Specimens collected in an Aptima Multitest Transport Tube do not require additional dilution with STM prior to testing.

   Note: *The recommended combined volume of each individual specimen is dependent upon the dimensions of tube being utilized. A Hologic representative can provide recommendations on minimum volume requirements for processing on the Panther system.*

C. Prior to testing on the Panther system, carefully transfer the determined volume of each individual specimen from the Aptima Multitest Transport Tubes to the empty tube.

D. Ensure homogeneous mixing of each prepared sample pool.

E. Retain the individual specimens for additional testing if required.

Panther System

Reagents for the Aptima SARS-CoV-2 assay are listed below for the Panther System. Reagent Identification Symbols are also listed next to the reagent name.

Reagents and Materials Provided

Aptima SARS-CoV-2 Assay Kit PRD-06419

250 tests (2 boxes)

Aptima SARS-CoV-2 Refrigerated Box (Box 1 of 2)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity 250 test kit
A	**Aptima SARS-CoV-2 Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**Aptima SARS-CoV-2 Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**Aptima SARS-CoV-2 Probe Reagent** *Non-infectious chemiluminescent DNA probes dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**Aptima SARS-CoV-2 Internal Control**	1 vial

Aptima SARS-CoV-2 Room Temperature Box (Box 2 of 2)
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity 250 test kit
AR	**Aptima SARS-CoV-2 Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 x 27.7 mL
ER	**Aptima SARS-CoV-2 Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 x 11.1 mL
PR	**Aptima SARS-CoV-2 Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 x 35.4 mL
S	**Aptima SARS-CoV-2 Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 x 108 mL
TCR	**Aptima SARS-CoV-2 Target Capture Reagent** *Buffered salt solution containing solid phase and capture oligomers.*	1 x 54 mL
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

	Cat. No.
Panther System	303095
Aptima Assay Fluids Kit *(Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)*	303014 (1000 tests)
Aptima Auto Detect Kit	303013 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther Run Kit *contains MTUs, waste bags, waste bin covers, assay fluids, and auto detects*	303096 (5000 tests)
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
Aptima SARS-CoV-2 Controls Kit *PC - Aptima SARS-CoV-2 Positive Control. Non-infectious nucleic acid in a buffered solution containing < 5% detergent. Quantity 5 x 1.7 mL* *NC - Aptima SARS-CoV-2 Negative Control. A buffered solution containing <5% detergent. Quantity 5 x 1.7 mL*	PRD-06420
Aptima Multitest Swab Specimen Collection Kit	PRD-03546
Aptima Specimen Transfer Kit	301154C
Aptima Specimen Transfer Kit - printable	PRD-05110
Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens	301041
Panther Fusion Specimen Lysis Tubes, 100 per bag *tube contains 0.71 mL of STM with a penetrable cap*	PRD-04339
Hologic Specimen Lysis Tube, 100 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06554
Hologic Specimen Lysis Tube, 1200 each *tube contains 0.71 mL of STM with a solid cap*	PRD-06660
Bleach, 5% to 7% (0.7M to 1.0M) sodium hypochlorite solution	—
Disposable gloves	—
Replacement non-penetrable caps	504415
Fisherbrand VersaClosure Tube Closures*, 1000 per pack **a single-use tube cover for the Hologic Specimen Lysis Tube (PRD-06554 only) after testing*	02-707

	Cat. No.
Replacement Caps for the 250-test kits	—
Amplification and Probe reagent reconstitution solutions	*CL0041 (100 caps)*
Enzyme Reagent reconstitution solution	*501616 (100 caps)*
TCR and Selection reagent	*CL0040 (100 caps)*

Optional Materials

	Cat. No.
Hologic Bleach Enhancer for Cleaning *for routine cleaning of surfaces and equipment*	302101
Tube rocker	—

Panther System Test Procedure

Note: *Refer to the Panther/Panther System Operator's Manual for additional procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Reconstitution/Preparation of a New Kit

Note: *Reagent reconstitution should be performed prior to beginning any work on the Panther System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the Panther System.*

Warning: *Adequate mixing of the reagents is necessary to achieve expected assay results.*

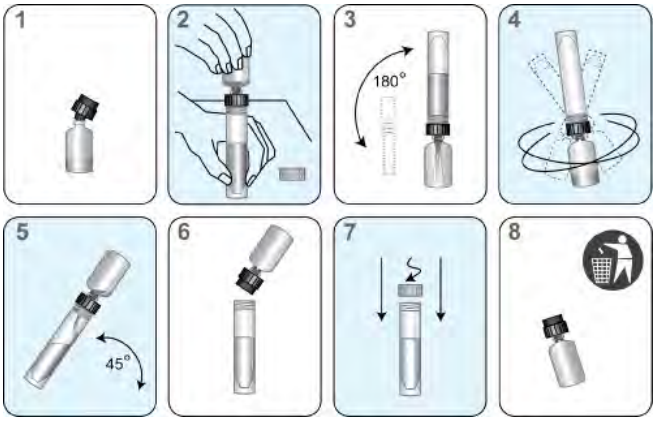

Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

3. Prepare Selection Reagent
   a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.
   b. Record operator initials and the current date on the label.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

   Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Probe Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.
3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.
4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.
5. *Adequate mixing of the reagents is necessary to achieve expected assay results.*

D. Specimen Handling using Panther Fusion Specimen Lysis Tube or Aptima Specimen Transfer Tube.

Note: *Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.*

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *For samples transferred to the Panther Fusion Specimen Lysis Tube or the Aptima Specimen Transfer Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.*

E. Specimen Handling using Hologic Specimen Lysis Tube

1. Prepare specimens per the Specimen Processing instructions in the *Specimen Collection and Storage* section.

Note: *For samples transferred to the Hologic Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis Tube (PRD-06554), there is sufficient volume to perform 2 nucleic acid extractions.*

Note: *When adequate collected specimen is added to the Hologic Specimen Lysis tube (PRD-06660), there is sufficient volume to perform 1 nucleic acid extraction.*

Note: *When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.*

F. System Preparation

1. Set up the system according to the instructions in the *Panther/Panther Fusion System Operator's Manual* and *Procedural Notes*. Make sure that the appropriately sized reagent racks and TCR adapters are used.
2. Load samples.

Procedural Notes

A. Controls

1. To work properly with the Aptima Assay software for the Panther System, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:
   a. A pair of controls is currently being processed by the system.
   b. Valid results for the controls are registered on the system.
2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:
   a. Controls results are invalid.
   b. The associated assay reagent kit is removed from the system.
   c. The associated assay reagent kit has exceeded stability limits.
3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

B. Temperature

Room temperature is defined as 15°C to 30°C.

C. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

D. Lab Contamination Monitoring Protocol for the Panther System

There are many laboratory-specific factors that may contribute to contamination, including testing volume, workflow, disease prevalence and various other laboratory activities. These factors should be taken into consideration when contamination monitoring frequency is being established. Intervals for contamination monitoring should be established based on each laboratory's practices and procedures.

To monitor for laboratory contamination, the following procedure may be performed using the Aptima Unisex Swab Specimen Collection Kit for Endocervical and Male Urethral Swab Specimens:

1. Label swab transport tubes with numbers corresponding to the areas to be tested.
2. Remove the specimen collection swab (blue shaft swab with green printing) from its packaging, wet the swab in the specimen transport medium (STM), and swab the designated area using a circular motion.
3. Immediately insert the swab into transport tube.
4. Carefully break the swab shaft at the score line; use care to avoid splashing of the contents.
5. Recap the swab transport tube tightly.
6. Repeat Steps 2 to 5 for each area to be swabbed.

E. If the results are positive, see *Interpretation of Results*. For additional Panther System-specific contamination monitoring information, contact Hologic Technical Support.

Quality Control

A run or specimen result may be invalidated by the Panther System if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for

SARS-CoV-2. The internal control must be detected in all samples that are negative for SARS-CoV-2 targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	IC Result	Interpretation
Neg	Valid	SARS-CoV-2 not detected.
POS	Valid	SARS-CoV-2 detected.
Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2.

Interpretation of Results for Pooled Samples

Negative: Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive: Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Invalid: Specimens with an invalid result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on the laboratory workflow and required result reporting time.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

E. Nasopharyngeal wash/aspirate or nasal aspirates and self-collected under supervision of or healthcare provider collected nasal and mid-turbinate nasal swabs are additional acceptable upper respiratory specimens that can be tested with the Aptima SARS-CoV-2 assay; however, performance with these specimen types have not been determined.

F. Nasopharyngeal wash/aspirate and nasal aspirates specimen types should not be pooled.

G. Sample pooling has only been validated using nasopharyngeal swab specimens.

Aptima SARS-CoV-2 Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Aptima SARS-CoV-2 assay was determined by testing serial dilutions of pooled negative clinical nasopharyngeal swab specimens spiked with inactivated cultured SARS-CoV-2 virus (USA-WA1/2020; BEI Resources; NR-52281). Ten replicates of each serial dilution were evaluated using each of two assay reagent lots across two Panther systems. The LoD was determined to be 0.01 $TCID_{50}$/mL and verified by testing an additional 20 replicates with one assay reagent lot. The LoD was also confirmed using saline, Liquid Amies and specimen transport medium (STM) swab collection media.

The analytical sensitivity of the Aptima SARS-CoV-2 assay was additionally evaluated using reference material from three commercial vendors. Serial dilutions of the reference material were made in STM and 20 or more replicates at each level were tested using each of two assay reagent lots across two Panther systems. The reference materials and the lowest dilution levels resulting in ≥ 95% detection are listed in Table 2.

Table 2: Analytical Sensitivity Evaluation of Commercial Reference Material

Vendor	Name	Reference #	Lot #	Analytical Sensitivity
ZeptoMetrix	SARS-CoV-2 External Run control	NATSARS(COV2)-ERC	324332	83 Copies/mL
SeraCare	AccuPlex SARS-Cov-2 Reference Material	0505-0126	10483977	83 Copies/mL
Exact Diagnostic	SARS-CoV-2 Standard	COV019	20033001	83 Copies/mL

Analytical Sensitivity with the Aptima Specimen Transfer Tube Workflow

The determined 0.01 $TCID_{50}$/mL analytical sensitivity (limit of detection) of the Aptima SARS-CoV-2 assay was confirmed using the Aptima Specimen Transfer tube specimen preparation workflow. Confirmation was performed using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) in negative clinical nasopharyngeal (NP) swab, saline, Liquid Amies and specimen transport medium (STM) swab collection media by testing 20 replicates with one reagent lot (Table 3).

Table 3: LoD Confirmation with the Aptima Specimen Transfer Workflow

Target	Matrix	N Valid	N Positive	% Positive	Avg kRLU	StdDev kRLU	%CV
Inactivated SARS-CoV-2 virus	NP Swab	20	20	100%	1063	61	5.8%
	STM	20	20	100%	1064	116	10.9%
	Saline	20	20	100%	1102	60	5.4%
	Liquid Amies	20	20	100%	1101	51	4.7%

Reproducibility

The Aptima SARS-CoV-2 assay reproducibility was evaluated on three Panther systems at a single site using four panel members. Testing was performed using three lots of assay reagents with two operators over six days. Two runs were performed per operator per day for a total of 36 runs. Each of the four panels was tested in three replicates per run for a total of 108 replicates per panel.

Positive and negative panel members consisted of pooled clinical nasopharyngeal (NP) swab matrix combined with Solution Transport Media (STM) in a ratio of 1:1.56. Positive panel members spiked with inactivated cultured SARS-CoV-2 virus at 0.1x LoD (High Negative), 1x LoD (Low Positive) and 5x LoD (Moderate Positive).

The agreement with expected results was 100% in the Negative, Low Positive and Moderate Positive panel members. The High Negative panel member was 10x below the assay LoD, therefore a mix of positive and negative results were expected. This panel gave 68/108 (63%) positive results. Agreement with expected results for all four panels is shown in Table 4.

Table 4: Agreement of Aptima SARS-CoV-2 Assay Results with Expected Results

Panel Description	Panel Composition	Panel Conc. $TCID_{50}$/mL	Expected Result	N Positive	N Tested	Mean kRLU	Agreement w/Expected (95% CI)
Negative	N/A	N/A	Negative	0	108	289	100% (96.6-100)
High Negative	0.1x LoD	0.001	N/A	68	108	627	N/A
Low Positive	1.0x LoD	0.01	Positive	108	108	1131	100% (96.6-100)
Moderate Positive	5.0x LoD	0.05	Positive	108	108	1147	100% (96.6-100)

The total SARS-CoV-2 signal variability measured as %CV ranged from 2.75% to 3.84% in Negative, Low Positive, and Moderate Positive panel members. For the sources of variation all six factors evaluated had %CV values <3.0% as shown in Table 5. The High Negative panel member is 10x below the assay LoD and the %CV for this panel is expected to be higher than the others. The highest source of variability for this panel was within-run variability.

Table 5: kRLU Signal Variability of the Aptima SARS-CoV-2 Assay by Panel Member

Panel	Between Days		Between Instruments		Between Operators		Between Lots		Between Runs		Within Runs		Total	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Negative	0.91	0.31	4.97	1.72	0.0	0.0	4.04	1.40	0.0	0.0	6.75	2.33	9.35	3.23
High Negative*	30.45	4.85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	244.08	38.91	245.97	39.21
Low Positive	6.46	0.57	6.74	0.60	0.0	0.0	28.10	2.48	0.0	0.0	31.77	2.81	43.43	3.84

Table 5: kRLU Signal Variability of the Aptima SARS-CoV-2 Assay by Panel Member

Moderate Positive	8.53	0.74	5.59	0.49	0.0	0.0	22.98	2.00	11.06	0.96	15.59	1.36	31.59	2.75

*Panel was built to 10x below the assay LoD. Higher variability is expected in this panel.

Inclusivity

The inclusivity of the Aptima SARS-CoV-2 assay was evaluated using *in silico* analysis of the assay target capture oligos, amplification primers, and detection probes in relation to 9,896 SARS-CoV-2 sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis, resulting in 9,879 sequences evaluated for the first target region of the assay and 9,880 for the second target region. The *in silico* analysis showed 100% homology to the assay oligos of both target systems for 9,749 (98.5%) of the evaluated sequences and 100% homology to the assay oligos of at least one target system for all 9,896 sequences. There were no evaluated sequences with identified mismatches predicted to impact binding or performance of both target systems.

Analytical Specificity and Microbial Interference

The analytical specificity of the Aptima SARS-CoV-2 assay was evaluated by testing 30 microorganisms representing common respiratory pathogens or closely related species (Table 6). Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. Microorganisms were tested with and without the presence of SARS-CoV-2 inactivated virus at 3x LoD. Analytical specificity of the Aptima SARS-CoV-2 assay was 100% with no evidence of microbial interference.

In addition to microorganism testing, *in silico* analysis was performed to assess the specificity of the assay in relation to the microorganisms listed in Table 6. The *in silico* analysis showed no probable cross reactivity to any of the 112 GenBank sequences evaluated.

Table 6: Aptima SARS-CoV-2 Analytical Specificity and Microbial Interference Microorganisms

Microorganism	Concentration	Microorganism	Concentration
Human coronavirus 229E	1E+5 $TCID_{50}$/mL	Parainfluenza virus 1	1E+5 $TCID_{50}$/mL
Human coronavirus OC43	1E+5 $TCID_{50}$/mL	Parainfluenza virus 2	1E+5 $TCID_{50}$/mL
Human coronavirus HKU1[1]	1E+6 copies/mL	Parainfluenza virus 3	1E+5 $TCID_{50}$/mL
Human coronavirus NL63	1E+4 $TCID_{50}$/mL	Parainfluenza virus 4	1E+3 $TCID_{50}$/mL
SARS-coronavirus[1]	1E+6 copies/mL	Influenza A	1E+5 $TCID_{50}$/mL
MERS-coronavirus	1E+4 $TCID_{50}$/mL	Influenza B	2E+3 $TCID_{50}$/mL
Adenovirus (e.g. C1 Ad. 71)	1E+5 $TCID_{50}$/mL	Enterovirus (e.g. EV68)	1E+5 $TCID_{50}$/mL
Human Metapneumovirus (hMPV)	1E+6 $TCID_{50}$/mL	Rhinovirus	1E+4 $TCID_{50}$/mL
Respiratory syncytial virus	1E+5 $TCID_{50}$/mL	*Legionella pneumophila*	1E+6 CFU/mL
Chlamydia pneumoniae	1E+6 IFU/mL	*Mycobacterium tuberculosis*	1E+6 $TCID_{50}$/mL
Haemophilus influenzae	1E+6 CFU/mL	*Streptococcus pneumoniae*	1E+6 CFU/mL
Bordetella pertussis	1E+6 CFU/mL	*Streptococcus pyogenes*	1E+6 CFU/mL
Pneumocystis jirovecii (PJP)	1E+6 nuc/mL	*Streptococcus salivarius*	1E+6 CFU/mL
Candida albicans	1E+6 CFU/mL	*Mycoplasma pneumoniae*	1E+6 CFU/mL
Staphylococcus epidermidis	1E+6 CFU/mL	*Pseudomonas aeruginosa*	1E+6 CFU/mL
Pooled human nasal wash[2] - *to represent diverse microbial flora in human respiratory tract*	N/A		

[1] Cultured virus and whole genome purified nucleic acid for Human coronavirus HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus IVTs corresponding to the ORF1ab gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

[2] In place of evaluating pooled human nasal wash, testing of 30 individual negative clinical NP swab specimens was performed to represent diverse microbial flora in the human respiratory tract.

Clinical Performance

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in comparison to the Panther Fusion SARS-CoV-2 assay (Hologic, Inc.) using a panel of remnant clinical specimens. For the study, remnant clinical nasopharyngeal specimens were collected from US patients with signs and symptoms of respiratory infection.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated in relation to the Panther Fusion assay as the reference result, as shown in Table 7. The Aptima SARS-CoV-2 assay showed positive and negative agreements of 100% and 98.2%, respectively.

Nasopharyngeal wash/aspirate, nasal aspirates, nasal swabs and mid-turbinate nasal swabs are acceptable specimens to test for viral respiratory infections. However, performance with these specimen types has not been specifically evaluated with the Aptima SARS-CoV-2 assay.

Table 7: Aptima SARS-CoV-2 Clinical Agreement

		Panther Fusion SARS-CoV-2 Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	50	1
	Negative	0	54

Positive Percent Agreement: (95% CI): 100% (92.9% – 100%)
Negative Percent Agreement: (95% CI): 98.2% (90.4% – 99.7%)
Overall Agreement: (95% CI): 99.0% (94.8% – 99.8%)

Clinical Performance with Contrived Panel

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to a panel of contrived specimens. For the study, a panel of 115 remnant clinical nasopharyngeal specimens was tested using both the Panther Fusion Specimen Lysis Tube (Specimen Lysis Tube) and Aptima Specimen Transfer tube workflows. All specimens were collected from US patients with signs and symptoms of respiratory infection. The panel consisted of 65 SARS-CoV-2 positive and 50 SARS-CoV-2 negative specimens. Of the 65 positive specimens, 40 were at concentrations 0.5-2x LoD and 25 were at concentrations 3-5x LoD using inactivated cultured SARS-CoV-2 virus (USA-QA1/2020; BEI Resources; NR-52281) as the target.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for both specimen preparation workflows were calculated in relation to the expected result of the contrived specimen panel, as shown in Table 8 for the Aptima Specimen Transfer Tube and Table 9 for the Specimen Lysis Tube. Detection characteristics for the contrived specimens were calculated by target concentration, as shown in Table 10. Both specimen preparation workflows showed 100% agreement for the evaluated panels.

Table 8: Performance of the Aptima Specimen Transfer Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Aptima Specimen Transfer Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 9: Performance of the Specimen Lysis Tube Workflow Relative to Expected Results

		Expected Result		
		Positive	Negative	Total
Specimen Lysis Tube Result	Positive	65	0	65
	Negative	0	50	50
	Total	65	50	115

Overall Agreement: 100% (96.8% – 100%)
Positive Agreement: 100% (94.4% – 100%)
Negative Agreement: 100% (92.9% – 100%)

Table 10: Detection Characteristics for Contrived Nasopharyngeal Swab Specimens

	Aptima Specimen Transfer Sample Workflow						Specimen Lysis Tube Sample Workflow					
Target Conc.	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**	**n Valid**	**n Positive**	**% Positive**	**Average kRLU**	**St Dev kRLU**	**%CV**
Neg	50	0	0	299	9.7	3.2	50	0	0	300	9.3	3.1
0.5x LoD	10	10	100	1050	208.5	19.9	10	10	100	1153	113.0	9.8
1.0x LoD	10	10	100	1176	102.1	8.7	10	10	100	1205	24.3	2.0
1.5x LoD	10	10	100	1222	31.6	2.6	10	10	100	1223	21.9	1.8
2.0x LoD	10	10	100	1225	22.6	1.8	10	10	100	1237	26.0	2.1
3.0x LoD	10	10	100	1228	13.6	1.1	10	10	100	1215	25.5	2.1
4.0x LoD	5	5	100	1238	16.7	1.4	5	5	100	1212	12.5	1.0
5.0x LoD	10	10	100	1237	18.2	1.5	10	10	100	1246	28.3	2.3

Clinical Performance with Naturally Infected Positive Specimens

The clinical performance of the Aptima SARS-CoV-2 assay using the Aptima Specimen Transfer tube specimen preparation workflow was evaluated in comparison to the Specimen Lysis Tube workflow tested with both the Aptima and Panther Fusion SARS-CoV-2 assays. For the study, three dilutions of 15 unique SARS-CoV-2 positive nasopharyngeal swab specimens were prepared and processed using both workflows. SARS-CoV-2 samples were previously determined to be positive using a non-Hologic molecular assay.

The positive percent agreement between the Aptima SARS-CoV-2 Assay using the Aptima Specimen Transfer Tube and the Specimen Lysis Tube workflows were 97.5% (87.1% – 99.6%) and 100% (91.0% – 100%), respectively, when compared to the Panther Fusion SARS-CoV-2 assay using the Specimen Lysis Tube workflow as reference. The positive percent agreement of the Aptima Specimen Transfer tube workflow was 95.0% (83.5% – 98.6%) when compared to the Specimen Lysis Tube workflow as reference.

Clinical Performance of Pooling up to 5 Specimens Prior to Testing

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in pools consisting of up to 5 specimens. For the study, a pool size of 5 specimens was evaluated and included positive and negative specimen pools. Each positive specimen pool consisted of one positive

specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. For the study, 50 positive and 20 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and included 20% low positive specimens. Specimens for inclusion in the clinical performance of pooling study were chosen based on Ct results obtained with the Panther Fusion SARS-CoV-2 assay. The Panther Fusion SARS-CoV-2 assay was used for this purpose because the Panther Fusion SARS-CoV-2 and Aptima SARS-CoV-2 assays have the same LoD when evaluated with the FDA reference panel (i.e., 600 NDU/mL). Low positive specimens included in the study were defined as having a Ct value within 1-2 Ct of the LoD of the Panther Fusion SARS-CoV-2 assay. Both the pooled and individual specimens were evaluated with the Aptima SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result, as shown in Table 11. All evaluated positive specimens yielded a positive result in the pool. Since the kRLU values for the Aptima assay do not correspond to target concentration, signal and in silico sensitivity analysis was not performed.

Table 11: Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	50	0	50
	Negative	0	20	20
	Total	50	20	70

Overall Agreement: 100% (94.8% – 100.0%)
Positive Agreement: 100% (92.9% – 100.0%)
Negative Agreement: 100% (83.9% – 100.0%)

Clinical Performance in Asymptomatic Individuals

The clinical performance of the Aptima SARS-CoV-2 assay in individuals without signs and symptoms of respiratory infection (asymptomatic individuals) was evaluated in comparison to an EUA molecular assay. Prospectively collected nasopharyngeal swab specimens from US patients were assessed, including 45 specimens positive for SARS-CoV-2 and 315 specimens negative for SARS-CoV-2 using the EUA comparator assay. The PPA and NPA were calculated in relation to the EUA comparator assay results. The PPA and NPA were 100% and 96.5%, respectively, for the Aptima SARS-CoV-2 assay in asymptomatic individuals, as shown in Table 12.

Table 12: Aptima SARS-CoV-2 Clinical Agreement

		EUA Assay	
		Positive	Negative
Aptima SARS-CoV-2 Assay	Positive	45	11
	Negative	0	304

Positive Percent Agreement (PPA): 100% (92.1% – 100.0%)
Negative Agreement (NPA): 96.5% (93.9% – 98.0%)

Six (6) of the 11 NP swab specimens with false positive results were confirmed positive following retesting with the comparator EUA assay. Ct values for these 6 samples ranged between 35.5 and 38.9, suggestive of low viral load.

Clinical Performance of Pooling up to 5 Asymptomatic Patient Specimens Prior to Testing

The clinical performance of the Aptima SARS-CoV-2 assay was evaluated in specimen pools with specimens collected from asymptomatic patients. Pool sizes of up to 5 specimens were evaluated with both positive and negative asymptomatic patient specimens. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. For a pool size of three, 32 positive and 32 negative specimen pools were evaluated. For a pool size of four, 36 positive and 31 negative specimen pools were evaluated. For a pool size of five, 36 positive and 30 negative specimen pools were evaluated. The positive specimens used in the study covered the detectable range of the assay and each pool size included 25% low positive specimens. Specimens included in the clinical performance study were chosen based on Ct results obtained with the Panther Fusion SARS-CoV-2 assay. The Panther Fusion SARS-Cov-2 assay was used for this purpose because the Panther Fusion SARS-CoV-2 and the Aptima SARS-CoV-2 assays have the same LoD when evaluated with the FDA reference panel (i.e. 600 NDU/mL). Low positive specimens included in the study were defined as having a Ct value within 1-2 Ct of the LoD of the Panther Fusion SARS-CoV-2 assay. Both the pooled and individual specimens were evaluated with the Aptima SARS-CoV-2 assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated in relation to the expected (individual) result for each evaluated pool size, as shown in Table 13, Table 14, and Table 15. With a pool size of three, one of the eight specimens evaluated with a target concentration at or near the LoD of the assay yielded an individual positive result but was not detected as part of a specimen pool. With a pool size of four, all evaluated positive specimens yielded a positive result when tested pooled. With a pool size of five, five of the nine specimens evaluated with target concentrations at or near the LoD of the assay yielded an individual positive result but were not detected as part of a specimen pool. Since the kRLU values for the Aptima assay do not correspond to target concentrations, signal and *in silico* sensitivity analysis was not performed.

Table 13: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 3

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 3 Result	Positive	31	0	31
	Negative	1	32	33
	Total	32	32	64

Overall Agreement: 98.4% (91.7% - 99.7%)
Positive Agreement: 96.9% (84.3% - 99.4%)
Negative Agreement: 100% (89.3% - 100%)

Table 14: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 4

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 4 Result	Positive	31	0	31
	Negative	5	30	35
	Total	36	30	66

Overall Agreement: 100% (94.6% - 100%)
Positive Agreement: 100% (90.4% - 100%)
Negative Agreement: 100% (89.0% - 100%)

Table 15: Asymptomatic Individual and Pooled Specimen Agreement with a Pool Size of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pool of 5 Result	Positive	31	0	31
	Negative	5	30	35
	Total	36	30	66

Overall Agreement: 92.4% (83.5% - 96.7%)
Positive Agreement: 86.1% (71.3% - 93.9%)
Negative Agreement: 100% (88.6% - 100%)

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cobas®

Rx Only

cobas® SARS-CoV-2 Qualitative

Nucleic acid test for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® SARS-CoV-2 Qualitative 192T	P/N: 09446109190
cobas® SARS-CoV-2 Qualitative 480T	P/N: 09448870190
cobas® SARS-CoV-2 Qualitative Control Kit	P/N: 09446117190
cobas® Buffer Negative Control Kit	P/N: 09051953190

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Intended use

cobas® SARS-CoV-2 Qualitative for use on the **cobas**® 5800/6800/8800 Systems is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in healthcare provider-instructed self-collected anterior nasal (nasal) swab and saliva specimens (collected on site), and healthcare provider-collected nasal, nasopharyngeal, and oropharyngeal swab specimens collected from any individuals, including those suspected of COVID-19 by their healthcare provider, and those without symptoms or other reasons to suspect COVID-19.

This test is also intended for the qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples containing up to and including six individual samples from healthcare provider-instructed self-collected nasal swab specimens (collected on site), or healthcare provider-collected nasal, nasopharyngeal, and oropharyngeal swab specimens. Negative results from pooled samples should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually. Specimens included in pools with a positive or presumptive positive result must be tested individually prior to reporting a result. Specimens with low SARS-CoV-2 RNA concentrations may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA but may not represent the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

cobas® SARS-CoV-2 Qualitative is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Summary and explanation of the test

Explanation of the test

cobas® SARS-CoV-2 Qualitative is a qualitative nucleic acid test for use on the **cobas**® 5800 System, **cobas**® 6800 System or **cobas**® 8800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in individual saliva specimens collected in a sterile empty collection container and individual or pooled nasal, nasopharyngeal and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), **cobas**® PCR Media, or 0.9% physiological saline. The RNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titer positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 Qualitative is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas®** 5800 System is designed as one integrated instrument. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 5800 System or **cobas®** 6800/8800 Systems software(s), which assigns test results for all tests. Results can be reviewed directly on the system screen, and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved by the use of non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The **cobas®** SARS-CoV-2 Qualitative master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labeled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

Reagents and materials

The materials provided for **cobas®** SARS-CoV-2 Qualitative can be found in Table 1. Materials required, but not provided can be found in Table 2, Table 3, Table 4, Table 8, Table 9, and Table 10.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® SARS-CoV-2 Qualitative reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 **cobas®** SARS-CoV-2 Qualitative

cobas® SARS-CoV-2 Qualitative Store at 2-8°C 192 test cassette (P/N 09446109190) 480 test cassette (P/N 09448870190)			
Kit components	**Reagent ingredients**	**Quantity per kit 192 tests**	**Quantity per kit 480 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from *Bacillus subtilis*. May produce an allergic reaction.	22.3 mL	38 mL
RNA Internal Control (RNA IC)	Tris buffer, < 0.05% EDTA, < 0.001% non-Sarbecovirus related armored RNA construct containing primer and probe specific primer sequence regions (non-infectious RNA in MS2 bacteriophage), < 0.1% sodium azide	21.2 mL	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL	14.5 mL
SARS-CoV-2 QL Master Mix Reagent 2 (SARS-CoV-2 QL MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream SARS-CoV-2 and Sarbecovirus primers, < 0.01% Internal Control forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for SARS-CoV-2, Sarbecovirus, and the RNA Internal Control, < 0.01% oligonucleotide aptamer, < 0.1% Z05D DNA polymerase, < 0.10% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL	17.5 mL

Table 2 cobas® SARS-CoV-2 Qualitative Control Kit

cobas® SARS-CoV-2 Qualitative Control Kit Store at 2–8°C (P/N 09446117190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
SARS-CoV-2 QL Positive Control (SARS-CoV-2 QL (+)C)	Tris buffer, < 0.05% Sodium azide, < 0.005% EDTA, 0.003% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing SARS-CoV-2 sequence, < 0.01% Non-infectious plasmid DNA (microbial) containing pan-Sarbecovirus sequence	16 mL (16 x 1 mL)

Table 3 cobas® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit Store at 2-8°C (P/N 09051953190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
cobas® Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P391: Collect spillage. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997563190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas®** SARS-CoV-2 Qualitative kits. See listing of additional materials required (Table 8 and Table 9).

** Product safety labeling primarily follows EU GHS guidance.

***Hazardous substance.

Reagent storage requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas®** 5800 or **cobas®** 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® SARS-CoV-2 Qualitative 192T	2–8°C
cobas® SARS-CoV-2 Qualitative 480T	2–8°C
cobas® SARS-CoV-2 Qualitative Control Kit	2–8°C
cobas® Buffer Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagent handling requirements for cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® SARS-CoV-2 Qualitative 192T	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® SARS-CoV-2 Qualitative 480T	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® SARS-CoV-2 Qualitative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas omni Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

*Single use reagents.

Time is measured from the first time that reagent is loaded onto the **cobas® 5800 System.

Reagent handling requirements for cobas® 6800/8800 Systems

Reagents loaded onto the **cobas®** 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas®** 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas®** 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas®** 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® SARS-CoV-2 Qualitative 192T	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours**
cobas® SARS-CoV-2 Qualitative 480T	Date not passed	90 days from first usage	Max 20 runs	Max 20 hours**
cobas® SARS-CoV-2 Qualitative Control Kit	Date not passed	Not applicable*	Not applicable	Max 8 hours**
cobas® Buffer Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 10 hours**
cobas omni Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

* Single use reagents.

** Time is measured from the first time that reagent is loaded onto the **cobas®** 6800/8800 Systems.

Additional materials required for cobas® 5800 System

Table 8 Materials and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas omni Processing Plate 24	08413975001
cobas omni Amplification Plate 24	08499853001
cobas omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300uL	07345607001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag or Solid Waste Bag With Insert	07435967001 or 08030073001
cobas omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050*,**	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR*,**	11902997001
16-position tube carrier*	09224319001
5-position rack carrier*	09224475001

*Contact your local Roche representative for a detailed order list for sample racks.

*,** MPA 16mm rack or 16-position tube carrier are the preferred racks for use with samples collected in **cobas*** PCR Media tubes. If RD5 racks are used, make sure to fill in the sample tubes with not less than the recommended minimum sample input. The tubes sit higher in an RD5 rack because of the rubber gasket at the bottom of each tube position. Therefore, it is possible that when using RD5 racks, the system could accept tubes that are below the minimum sample input volume and cause pipetting errors later in the run.

Additional materials required for cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001
cobas omni Secondary Tubes 13x75 (optional)	06438776001
cobas® PCR Media Tube Replacement Cap Kit	07958056190
cobas® PCR Media Disposable Tube Stand (Optional)	07958064190
MPA RACK 16 MM LIGHT GREEN 7001-7050***	03143449001
RD5 RACK – RD Standard rack 0001-0050 LR***	11902997001

* MPA 16 mm or RD5 racks are required to use **cobas**® SARS-COV-2 Qualitative. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

MPA 16 mm rack is the preferred rack for use with samples collected in **cobas® PCR Media tubes. If RD5 racks are used, make sure to fill in the sample tubes with not less than the recommended minimum sample input. The tubes sit higher in an RD5 rack because of the rubber gasket at the bottom of each tube position. Therefore, it is possible that when using RD5 racks, the system could accept tubes that are below the minimum sample input volume and cause pipetting errors later in the run.

Alternative collection kits for swab specimens for use on the cobas® 5800/6800/8800 Systems

Table 10 Alternative specimen collection kits used with **cobas**® SARS-CoV-2 Qualitative for swab specimens

Collection Kit	P/N
cobas® PCR Media Uni Swab Sample Kit	07958030190
cobas® PCR Media Dual Swab Sample Kit	07958021190
cobas® PCR Media 100 tube kit	06466281190
cobas® Uni Swab 100 Kit	09205098190

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® SARS-CoV-2 Qualitative analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

The **cobas**® 6800/8800 Systems software and **cobas**® SARS-CoV-2 Qualitative analysis package for the **cobas**® 6800/8800 Systems must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 11 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
cobas® 6800 System (Moveable Platform)	05524245001 or 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 or 06379664001
cobas® 8800 System	05412722001
Sample Supply Module (**cobas**® 6800/8800 Systems only)	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guide for additional information.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[1,2] Only personnel proficient in handling infectious materials and the use of **cobas**® SARS-CoV-2 Qualitative and the **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- **Some positive samples may not be detected when diluted and tested in pools.** SARS-CoV-2 RNA concentration is reduced when a positive sample is pooled with other samples, and the reduction corresponds inversely to the pool size. For example, if there is only one positive sample in a pool of 6, the concentration in the original sample would need to be 6 times the assay limit of detection in order for the concentration in the pool to be at the limit of detection.
- Inform your local competent authority about any serious incidents which may occur when using this assay.
- Reliable saliva results are dependent on proper specimen collection, handling, and storage. In case of visible particles or discoloration in the saliva sample, samples should not be further processed, but patient shall be asked to provide a new sample. Food particles and increased levels of mucin might cause failures in processing of the saliva specimen.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.

- **cobas**® SARS-CoV-2 Qualitative, **cobas**® SARS-CoV-2 Qualitative Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® SARS-CoV-2 Qualitative kits, **cobas**® SARS-CoV-2 Qualitative Control Kit, **cobas**® Buffer Negative Control Kit and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800 or **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® Systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of the instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Sample collection – swab specimen types

Ensure that the correct collection device is used with the appropriate sample type by referring to the table below:

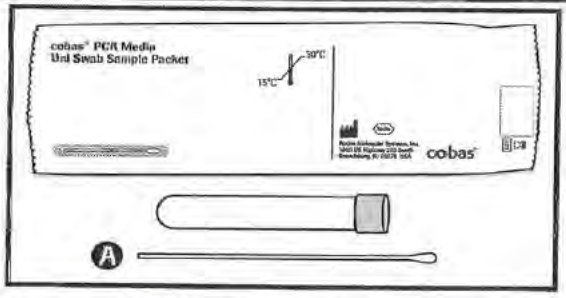
Table 12 Overview of collection devices and sample types

Collection Device	Sample Type		
	Nasopharyngeal	Oropharyngeal	Nasal
Copan Universal Transport Media (UTM-RT®)	√	√	√
BD™ Universal Viral Transport (UVT)	√	√	√
cobas® PCR Media Uni Swab Sample Kit			√
cobas® PCR Media Dual Swab Sample Kit			√
cobas® PCR Media Kit (and 100 tube PCR Media Kit)			√
0.9% Physiological saline			√

- Collect nasal, nasopharyngeal and oropharyngeal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of Copan Universal Transport Medium (UTM-RT) or BD™ Universal Viral Transport (UVT).
- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place into **cobas®** PCR Media tube from **cobas®** PCR Media Kit (P/N 06466281190).
- Collect nasal specimens using the **cobas®** PCR Media Uni Swab Sample Kit (P/N 07958030190) or **cobas®** PCR Media Dual Swab Sample Kit (P/N 07958021190) according to instructions below.
- Refer to the Instructions for Use of the Collection Devices for hazard information.

Nasal (anterior nares) swab specimen collection - clinician or self-collected on site

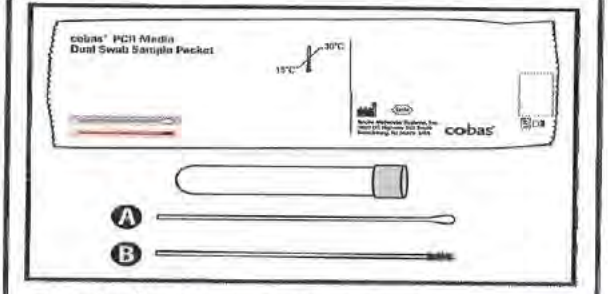
WARNING: DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!



The **cobas®** PCR Media Uni Swab Sample kit contains:

cobas® PCR Media tube
Woven Swab: A

OR



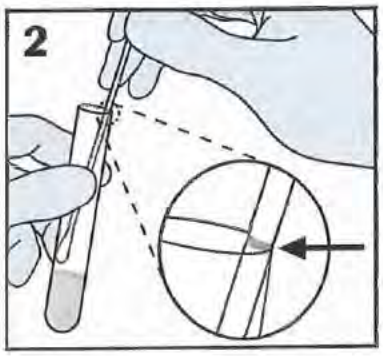
The **cobas®** PCR Media Dual Swab Sample kit contains:

cobas® PCR Media Tube
Woven Swab: A
Flocked Swab: B

DO NOT PRE-WET SWAB IN cobas® PCR MEDIA BEFORE COLLECTION!

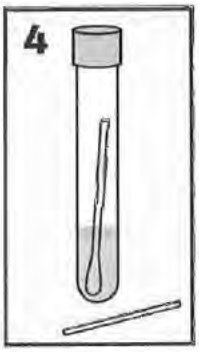
1. **COLLECT:** Hold the woven swab (Swab A) or the flocked swab (Swab B) with the scoreline above your hand. Insert the swab 1-2 cm into one of the anterior nares. Rotate the swab against the nasal mucosa for about 3 seconds and withdraw. Repeat with the other anterior nare using the same swab.

   Do not let the swab touch any surface before placing it into the collection tube.



2. **ALIGN:** Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim.

3. **BREAK:** Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.



4. **CLOSE: Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

- Collect nasal specimens according to standard collection technique using flocked or polyester-tipped swabs and immediately place in 3 mL of 0.9% physiological saline.

Sample collection – saliva

- Do NOT eat or brush teeth 60 minutes before collecting the saliva sample.
- Collect saliva on the floor of your mouth and allow to pool without swallowing. Do not cough.
- Spit passively collected saliva into a sterile collection container.
- Repeat the procedure above until at least 1 mL, but no more than 5 mL of saliva are collected in the container.
- Replace cap on the saliva collection container.
- Return the saliva collection container.

Raw saliva needs to be liquified within 9 days of collection (48 hours at 2-25°C followed by 7 days below -18°C) by estimating the volume of saliva and adding double the amount of 0.9% physiological saline solution to the saliva collection container. After addition of saline, the sample needs to be mixed (e.g., vortex 10 – 20 seconds) prior to further storage or processing.

Transport and storage – swab specimen types

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Samples collected in UTM-RT®,
  - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.
- Samples collected in **cobas**® PCR Media,
  - After collection, specimens can be stored for up to 24 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.
- Samples collected in 0.9% physiological saline,
  - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 3 days at 2-8°C and at ≤ -70°C for up to 30 days.
- Specimen are stable for up to two freeze/thaw cycles when frozen at ≤ -70°C.

Transport and storage – saliva

- Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.
- Raw saliva samples collected in a sterile polypropylene collection device,
  - After collection, specimens can be stored for up to 48 hours at 2-25°C followed by up to 7 days ≤ -18°C.
- Liquified saliva,
  - After addition of 2 parts of 0.9% physiological saline solution and intensive mixing, the liquefied saliva specimens can be stored for up to 48 hours at 2-25°C followed by up to 7 days at 2-8°C.

Instructions for use

Procedural notes

- Do not use **cobas**® SARS-CoV-2 Qualitative, **cobas**® SARS-CoV-2 Qualitative Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of the sample racks. Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems User Guide for proper barcode specifications and additional information on loading sample tubes.
- Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for proper maintenance of instruments.

Running cobas® SARS-CoV-2 Qualitative utilizing swab specimens

For testing swab specimens, **cobas**® SARS-CoV-2 Qualitative can be run with a minimum required sample volume of 0.6 mL in the **cobas omni** Secondary Tube for specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), **cobas**® PCR Media or 0.9% physiological saline. Specimens collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kit can be run in their primary collection tube with a minimum required sample volume of 1.0 mL.

Specimens collected in cobas® PCR Media, 0.9% physiological saline, UTM-RT or UVT

Specimens collected in tubes compatible with the **cobas**® 5800 and **cobas**® 6800/8800 Systems may be loaded directly onto the **cobas**® 5800 and **cobas**® 6800/8800 Systems. Specimens collected in Copan Universal Transport Medium (UTM-RT), BD™ Universal Viral Transport (UVT), **cobas**® PCR Media or 0.9% physiological saline tubes which are not compatible with the **cobas**® 5800 and **cobas**® 6800/8800 Systems must be transferred into a secondary tube prior to processing on the **cobas**® 5800 and **cobas**® 6800/8800 Systems. The **cobas omni** Secondary Tube is the preferred option. Samples should be processed using the sample type selection in the user interface (UI) as described in Table 13. Additional tubes for testing **cobas**® SARS-CoV-2 Qualitative are available. Contact your local Roche representative for detailed testing instructions and an order list of primary tubes and secondary tubes compatible with the instruments.

Always use caution when transferring specimens from a primary collection tube to a secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni Secondary Tube.

Follow the steps below to transfer patient sample from a primary collection tube into a **cobas omni** Secondary Tube:

- Unscrew the primary sample tube cap.
- Lift the cap and any attached swab to allow a pipette to be inserted into the sample tube. ·
- Transfer 0.6 mL into the prepared barcoded secondary tube. ·
- Transfer secondary tube to a rack. Close the primary sample tube cap.

Specimens collected using cobas® PCR Media Uni or Dual Swab Sample Kit or cobas® PCR Media together with cobas® Uni Swab 100 Kit

Samples collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kit or **cobas**® PCR Media together with the **cobas**® Uni Swab 100 Kit must be uncapped and can be loaded directly onto racks for processing on the **cobas**® 5800/6800/8800 Systems. Transfer into a secondary tube is not necessary. **cobas**® PCR Media tubes fit on to the MPA RACK 16 MM LIGHT GREEN 7001-7050 (P/N 03143449001) or the 16-position tube carrier (P/N 09224319001) and can be processed with the swab remaining in the tube. Samples collected using **cobas**® PCR Media Uni Swab Sample Kit or **cobas**® PCR Media Dual Swab Sample Kits or **cobas**® PCR Media together with the **cobas**® Uni Swab 100 Kit should be processed using the '**cobas**® PCR media swab' sample type selection in the user interface (UI) of the **cobas**® SARS-CoV-2 Qualitative as described in Table 13.

A properly collected swab specimen should have a single swab with the shaft broken at the scoreline. Swab shafts which are broken above the score line will appear longer than normal and may also be bent over to fit into the **cobas**® PCR Media tube. This may create an obstruction to the pipetting system which might cause the loss of sample, test results and/or mechanical damage to the instrument. In the event that a swab specimen has an improperly broken shaft, remove the swab prior to sample processing on the **cobas**® 5800/6800/8800 Systems. Use caution when disposing of specimen swabs; avoid splashing or touching swabs to other surfaces during disposal to prevent contamination.

Incoming **cobas**® PCR Media primary swab specimen tubes with no swabs or with two swabs have not been collected according to the instructions in their respective collection kit instruction for use and should not be tested. If the sample containing two swabs in the **cobas**® PCR Media primary tubes must be tested, transfer 0.6 mL into the prepared barcoded secondary tube.

Occasionally, incoming swab specimens contain excessive mucus which may induce a pipetting error (e.g., clot or other obstruction) on the **cobas**® 5800/6800/8800 Systems. Prior to retesting of specimens that exhibited clots during initial processing, remove and discard the swab, then re-cap and vortex these specimens for 30 seconds to disperse the excess mucus.

Swab specimens can be processed twice on the **cobas**® 5800/6800/8800 Systems while the swab is in the collection tube. If additional testing is required, or if the first test fails due to specimen pipetting error (e.g., clot or other obstruction), the swab must be removed and the remaining fluid must have a minimum volume of 1.0 mL.

Running cobas® SARS-CoV-2 Qualitative utilizing saliva specimens

Raw saliva specimens collected in a sterile polypropylene container need to be liquified prior to processing. For liquifaction, the volume of raw saliva is estimated and the double amount of 0.9% physiological saline is added. The collection device must be recapped and the solution mixed (e.g., vortex 10 – 20 seconds) prior to the required transfer into a secondary tube and processing on the **cobas**® 5800/6800/8800 Systems. The **cobas omni** Secondary Tube is the preferred option. Liquified saliva samples transferred to secondary tubes should be processed using the 'Saliva' sample type selection in the user interface (UI) of the **cobas**® SARS-CoV-2 Qualitative.

Always use caution when transferring specimens from a primary collection container to the secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to liquifaction and transfer into a secondary tube.

Follow the steps below to transfer saliva samples from a primary collection container into a secondary tube:

- Unscrew the primary sample container cap and lift the cap.
- Estimate the volume of raw saliva and add the double amount of 0.9% physiological saline.
- Recap the container and mix (e.g., vortex 10-20 seconds) until a homogenous solution results.
- Unscrew the primary sample container cap and lift the cap.
- Transfer 1.2 mL into the prepared barcoded secondary tube.
- Close the primary sample container cap.
- Transfer secondary tube to the rack.

Table 13 Sample type selection in the user interface of the **cobas®** SARS-CoV-2 Qualitative

Collection kit/Matrix type	**Minimum volume (mL) Processing tube**	**Process as Sample Type**
Copan Universal Transport Medium BD™ Universal Viral Transport 0.9% physiological saline **cobas®** PCR Media Kit	0.6 mL **cobas omni** Secondary Tube	**VTM**
cobas® PCR Media Uni or Dual Swab Sample Kit **cobas®** PCR Media Kit together with the **cobas®** Uni Swab 100 Kit	1.0 mL Primary tube	**cobas® PCR media swab**
Liquified Saliva in a sterile polypropylene container	1.2 mL **cobas omni** Secondary Tube	**Saliva**

Sample pooling for SARS-CoV-2 testing

Pools of up to and including 6 samples may be tested using **cobas®** SARS-CoV-2 Qualitative. The pool size implemented by the laboratory should be based on the required efficiency gains, the positivity rate of SARS-CoV-2 in the testing population, and the potential risks of testing in pools. Combination of multiple sample types in a pool has not been validated.

When resource availability is sufficient to meet testing demand, it is recommended that laboratories consider whether the risks of reduced test sensitivity with pooling outweigh the benefits of resource conservation.

- Use a process that ensures traceability between individual sample IDs and pool IDs.
- To reduce potential contamination of the **cobas®** 5800/6800/8800 Systems, do not transfer samples into the secondary tubes while the samples are in the Roche 5 position racks (RD5 and/or MPA and /or 16 position tube carrier).
- Ensure appropriate sample handling techniques to reduce the risk of cross-contamination of pools and original patient samples.

Note: Sample pooling does not apply to saliva specimen.

Pooling methods

1. Identify a uniquely labeled secondary tube for pooling.
2. Associate the samples to be pooled with the pool tube identification using either a pooling worksheet or validated sample tracking system.
3. Roche suggests using Biological Safety Cabinet or other approved safety measures during sample handling (i.e., sample transfer to secondary tube).
4. For manual pooling, it is recommended to work with only the samples for one pool at a time.
5. Ensure each sample has sufficient volume for pool construction and any possible resolution testing (pool deconvolution) that may be required. Example: for pools of 6, a minimum volume of 100 µL (for pool) plus 600 µL (for resolution) are required for a minimum sample volume of 700 µL available prior to pooling (Table 14).

Table 14 Minimum sample volumes for pooling

Pool Size	Volume required for pool (mL)	Volume required for resolution testing (mL)	Minimum volume required prior to pooling (mL)
6	0.100	0.600	0.700
5	0.120	0.600	0.720
4	0.150	0.600	0.750
3	0.200	0.600	0.800
2	0.300	0.600	0.900

6. Using a calibrated micropipettor with a fresh pipette tip for each sample, carefully pipette each individual sample associated with that pool into the appropriate secondary tube to prepare the pool.
7. Ensure complete mixing after addition of all samples to the secondary tube (i.e., through pipetting up and down). Use caution to avoid creating bubbles, foam or aerosols while mixing.
8. For manual pooling, it is recommended to visually compare the pooled sample volume in the secondary tube to a secondary tube containing the target pool volume. If the pooling tube level is less or more than the standard pool volume, then the manually prepared pool should be discarded and prepared again.
9. Process pooled samples as described in Figure 1 and Figure 2.

Pool result reporting and follow-up testing

Interpretation of pool results is the same as for individual results as described in the **Interpretation of results** section.

- If the result of the pool is negative, then each constituent sample can be reported as negative. The result report should include a comment that pooling was used during testing. Refer to the **Warnings and precautions** section for additional information regarding decreased sensitivity of pool testing.
- If the result of the pool is positive or presumptive positive, then each of the constituent samples must be re-tested as a separate individual sample. Use the laboratory defined tracking system to ensure the correct individual samples are tested. Individual test results supersede the pool result.

Running cobas® SARS-CoV-2 Qualitative on cobas® 5800 System

The test procedure is described in detail in the **cobas®** 5800 Systems User Assistance and/or User Guide. Figure 1 below summarizes the procedure.

Figure 1 **cobas®** SARS-CoV-2 Qualitative test procedure on the **cobas®** 5800 System

1	Log onto the system
2	Loading samples onto the system • Load sample racks onto the system • The system prepares automatically • Order tests
3	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette(s) • Load control mini racks • Load processing tips • Load elution tips • Load processing plates • Load liquid waste plates • Load amplification plates • Load MGP cassette • Refill specimen diluent • Refill lysis reagent • Refill wash reagent
4	Start the run by choosing the Start processing button on the user interface, all subsequent runs will start automatically if not manually postponed
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up the instrument • Unload empty control mini racks • Unload empty test specific reagent cassette(s) • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Running cobas® SARS-CoV-2 Qualitative on cobas® 6800/8800 Systems

The test procedure is described in detail in the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

Figure 2 **cobas**® SARS-CoV-2 Qualitative procedure on the **cobas**® 6800/8800 Systems

1. Log onto the system
   Press Start to prepare the system
   Order tests
2. Refill reagents and consumables as prompted by the system
   - Load test specific reagent cassette
   - Load control cassettes
   - Load pipette tips
   - Load processing plates
   - Load MGP reagent
   - Load amplification plates
   - Refill specimen diluent
   - Refill lysis reagent
   - Refill wash reagent
3. Loading samples onto the system
   - Load sample racks and clotted tip racks onto the sample supply module
   - Confirm samples have been accepted into the transfer module
4. Start the run by choosing the Start manually button on the user interface or have it start automatically after 120 minutes or if the batch is full
5. Review and export results
6. Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use

   Clean up the instrument
   - Unload empty control cassettes
   - Empty amplification plate drawer
   - Empty liquid waste
   - Empty solid waste

Results

The **cobas**® 5800/6800/8800 Systems automatically detect the SARS-CoV-2, for each individually processed sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Quality control and validity of results on the cobas® 5800 System

- One **cobas**® Buffer Negative Control [BUF (-) C] and one positive control [SARS-CoV-2 QL (+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 System software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with "Invalid" in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with "Invalid" show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 System software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 System software and/or report. The result interpretation should be as follows:

Table 15 Example of **cobas**® SARS-CoV-2 Qualitative results display **cobas**® 5800 System

Sample ID*	Test	Control Result	Flags**	Status	Result		Creation date/time
Sample_01	SCoV2-QL	Valid		Released	SCoV2 Negative	PanSarb Negative	7/7/2021 8:27:39 AM
Sample _C1	SCoV2-QL	Invalid		Released	Invalid	Invalid	7/7/2021 8:27:39 AM
Sample _B1	SCoV2-QL	Valid		Released	SCoV2 Negative	PanSarb Positive	7/7/2021 8:27:39 AM
Sample _B2	SCoV2-QL	Valid		Released	SCoV2 Positive	PanSarb Positive	7/7/2021 8:27:39 AM

Sample _D1	SCoV2-QL	Valid		Released	SCoV2 Negative	PanSarb Negative	7/7/2021 8:27:39 AM
Sample _A6	SCoV2-QL	Valid		Released	SCoV2 Positive	PanSarb Negative	7/7/2021 8:27:39 AM
Sample _E1	SCoV2-QL	Valid		Released	SCoV2 Positive	Invalid	7/7/2021 8:27:39 AM
Sample _A2	SCoV2-QL	Valid		Released	Invalid	PanSarb Positive	7/7/2021 8:27:39 AM

*Table applies for all sample types used.

** The result overview shows a flag symbol in case of invalid results. Detailed flag descriptions are available in the result details.

- Samples associated with a valid control batch are shown as "Valid" in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if Control Results are reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
  - Q05D: Result validation failure because of an invalid positive control.
  - Q06D: Result validation failure because of an invalid negative control.
- The values in "Result" column for individual sample target result should be interpreted as show in Table 15 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One **cobas**® Buffer Negative Control [BUF (-) C] and one Positive Control [SARS-CoV-2 QL (+)C] are processed with each batch.
- In the **cobas**® 6800/8800 Systems software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch.

Validation of results is performed automatically by the **cobas**® 6800/8800 Systems software based on negative and positive control performance.

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- Results display examples for **cobas**® SARS-CoV-2 Qualitative are shown in Table 16.
- The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas**® SARS-CoV-2 Qualitative.
- Invalid results for one or more target combinations are possible and are reported out specifically for each target. If any individual target result is invalid, the presence or absence of that individual target cannot be determined.
- Other initial valid target results can be interpreted as described in Table 17.

Table 16 Example of **cobas®** SARS-CoV-2 Qualitative results display on **cobas®** 6800/8800 Systems

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SCoV2- QL	Sample _01	NA		VTM	NA	SCoV2 Negative	PanSarb Negative
SCoV2- QL	Sample _C1	NA	Y40T	VTM	NA	Invalid	Invalid
SCoV2- QL	Sample _B1	NA		VTM	NA	SCoV2 Negative	**PanSarb Positive**
SCoV2- QL	Sample _B2	NA		VTM	NA	**SCoV2 Positive**	**PanSarb Positive**
SCoV2- QL L	Sample _D1	NA		VTM	NA	SCoV2 Negative	PanSarb Negative
SCoV2- QL	Sample _A6	NA		VTM	NA	**SCoV2 Positive**	PanSarb Negative
SCoV2- QL	Sample _E1	NA	C01H2	VTM	NA	**SCoV2 Positive**	Invalid
SCoV2- QL	Sample _A2	NA	C01H1	VTM	NA	Invalid	**PanSarb Positive**
SCoV2- QL	C161420284090428828404	Yes		(-) Ctrl	Valid	Valid	Valid
SCoV2- QL	C161420284093009580264	Yes		SCoV2-QL (+) C	Valid	Valid	Valid

The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas® SARS-CoV-2 Qualitative. Refer to Table 17, **cobas®** SARS-CoV-2 Qualitative results interpretation, for specific instructions on test results interpretation.

Table 17 cobas® SARS-CoV-2 Qualitative results interpretation

Target 1	Target 2	Interpretation
SCoV2 Positive	**PanSarb Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
SCoV2 Positive	**PanSarb Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
SCoV2 Negative	**PanSarb Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
SCoV2 Negative	**PanSarb Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
SCoV2	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**PanSarb Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
SCoV2 Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**PanSarb Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid.* Sample should be retested. If the result is still invalid, a new specimen should be obtained.

*For further details also refer to section **Procedural limitations**

Procedural limitations

- **cobas®** SARS-CoV-2 Qualitative has been evaluated only for use in combination with the **cobas®** SARS-CoV-2 Qualitative Control Kit, **cobas®** Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas®** 5800/6800/8800 Systems.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Due to the nature of the saliva sample type and the individual patient sample variability, an increased invalid and clot rate may occur. Additionally, food particles and increased levels of mucin indicated by potentially discolored samples might cause failures in processing of the saliva specimen. Refer to the **Sample collection – saliva** section for the appropriate precautions to be taken during collection to assure optimal performance.
- Occasionally, incoming saliva specimens may induce a pipetting error (e.g. clot or other obstruction) on the **cobas®** 5800/6800/8800 Systems. A potential additional processing step prior to retesting the specimens that exhibited clots during initial processing is to centrifuge the samples at 2000g for 1 minute and reload the samples on the instrument. Aerosols might occur during centrifugation. To avoid any contamination or transmission of the virus, please handle centrifuged samples carefully .
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasal, nasopharyngeal and oropharyngeal swab samples collected in a Copan UTM-RT System (UTM-RT) or BD™ Universal Viral Transport System (UVT) and nasal swab samples collected in **cobas®** PCR Media and 0.9% physiological saline. Additionally the test is intended to be used for the detection of SARS-CoV-2 RNA in saliva specimens liquified with 0.9% physiological saline. Testing of other sample types with **cobas®** SARS-CoV-2 Qualitative may result in inaccurate results.
- As with any molecular test, mutations within the target regions of **cobas®** SARS-CoV-2 Qualitative could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- False negative or invalid results may occur due to interference. The Internal Control is included in **cobas®** SARS-CoV-2 Qualitative to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas®** SARS-CoV-2 Qualitative Master Mix reagent enables selective amplification of target RNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents.

Use of pooling based on prevalence

Pooling may increase throughput in laboratories testing samples from populations with low prevalence of SARS-CoV-2. In populations with higher prevalence, smaller pool sizes or individual sample testing may be indicated.

When considering pooling strategies, laboratories should consider the appropriateness of the pooling strategy based on the positivity rate in the testing population and efficiency of the pooling workflow. Laboratories may also consider the sensitivity of pooled testing based on the assay's limit of detection.

Table 18 provides estimated maximal efficiency gained based on N-sample pooling and on the percent of SARS-CoV-2 positive samples in a population.

Table 18 Efficiency of pooling based on prevalence

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency (F) of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)
1% - 4%	6	4.44 - 2.60
5% - 6%	6	2.32 - 2.10
7% - 12%	6	1.92 - 1.42
13% - 25%	6	1.36 - 1.01
1% - 4%	5	4.02 - 2.60
5% - 6%	5	2.35 - 2.15
7% - 12%	5	1.98 - 1.49
13% - 25%	5	1.43 - 1.04
1% - 4%	4	3.46 - 2.50
5% - 6%	4	2.30 - 2.13
7% - 12%	4	1.99 - 1.54
13% - 25%	4	1.48 - 1.07
1% - 4%	3	2.75 - 2.23
5% - 6%	3	2.10 - 1.99
7% - 12%	3	1.89 - 1.53
13% - 25%	3	1.48 - 1.10
1% - 4%	2	1.92 - 1.73
5% - 6%	2	1.67 - 1.62
7% - 12%	2	1.57 - 1.38
13% - 25%	2	1.35 - 1.07

Because a positive pool requires individual retesting of each sample in the pool, the efficiency of any pooling strategy depends on the positivity rate. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated using the following formula $F=1/(1+1/n-(1-P)n)$. The efficiency (F) indicates how many more samples can be tested with n-sample pools compared to individual testing. For example, a 6-sample pooling strategy increases the number of tested samples by 2.10 times for positivity rate P of 6% (F = 2.10). At F = 2.10, 1,000 tests can cover testing of 2,100 samples on average.

Non-clinical performance evaluation

Analytical sensitivity (Limit of Detection) – swab specimen types

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive.

To determine the LoD, the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was serially diluted in simulated clinical matrix. A total of 5 concentration levels, and 3 independent dilution series, were tested with a total of 24 replicates per concentration and lot, with an additional 60 replicates of a blank sample (i.e., clinical sample pools).

As shown in Table 19 and Table 20, the concentration level with observed hit rates greater than or equal to 95% were 250 and 125 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively and the Probit predicted 95% hit rates were 200 and 102 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 19 Summary of LoD for SARS-CoV-2 using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**202**	157-319	**250**	33.2
	Lot 2	**121**	97-183	**125**	34.1
	Lot 3	**259**	196-413	**250**	33.2
	Lot 1-3	**200**	170-252	**250**	33.4

Table 20 Summary of LoD for pan-Sarbecovirus using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**83**	64-127	**125**	35.2
	Lot 2	**67**	46-454	**125**	36.0
	Lot 3	**132**	99-233	**125**	34.8
	Lot 1-3	**102**	83-140	**125**	35.3

Further, the sensitivity of the assay was determined using a cultured virus of an isolate from a US patient (USA-WA1/2020, catalog number NR-52281, lot number 70033175, 2.8E+05 $TCID_{50}$/mL[$]) was serially diluted in simulated clinical matrix. A total of 7 concentration levels, with 3-fold serial dilutions between the levels, were tested with a total of 21 replicates per concentration, with an additional 10 replicates of a blank sample (i.e., simulated clinical matrix).

As shown in Table 21 the concentration level with observed hit rates greater than or equal to 95% were 0.009 and 0.003 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively. As shown in Table 22, the Probit predicted 95% hit rates were 0.007 and 0.004 $TCID_{50}$/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 21 LoD determination using USA-WA1/2020 strain

Strain	Concentration [$TCID_{50}$/mL]	Total valid results	Hit rate [%]**		Mean Ct*	
			Target 1	Target 2	Target 1	Target 2
USA-WA1/2020* (stock concentration 2.8E+05 $TCID_{50}$/mL) Lot 70033175***	0.084	21	100	100	31.0	33.0
	0.028	21	100	100	31.8	34.1
	0.009	21	100	100	32.7	35.2
	0.003	21	38.1	100	33.5	36.4
	0.001	21	0	52.4	n/a	37.9
	0.0003	21	0	14.3	n/a	37.2
	0.0001	21	0	9.5	n/a	38.5
	0 (blank)	10	0	0	n/a	n/a

* The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281

** All replicates where Target 1 was positive were also positive for Target 2.

*** Based on the information provided in the Certificate of Analysis from the vendor, 1 TCID50/mL is equal to 7,393 genome equivalents by ddPCR

Table 22 Probit Predicted 95% Hit Rates Using USA-WA1/2020 Strain

Strain	Probit Predicted 95% Hit Rate [$TCID_{50}$/mL]	
	Target 1	Target 2
USA-WA1/2020 (stock concentration 2.8E+05 $TCID_{50}$/mL)	0.007 (95% CI: 0.005 – 0.036)	0.004 (95% CI: 0.002 – 0.009)

Analytical sensitivity (Limit of Detection) – saliva specimen types

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates tested positive.

To determine the LoD, the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) was serially diluted in pools of negative Saliva clinical specimens. A total of 8 concentration levels and 3 independent dilution series/saliva pools, were tested with a total of 32 replicates per concentration and lot, with an additional 96 replicates of a blank sample (i.e., clinical sample pools).

As shown in Table 23 and Table 24, the concentration levels with observed hit rates greater than or equal to 95% were 150 IU/mL and 75 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively and the Probit predicted 95% hit rates were 92 and 72 IU/mL for SARS-CoV-2 (Target 1) and pan-Sarbecovirus (Target 2), respectively.

Table 23 Summary of LoD for SARS-CoV-2 using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**102**	76-156	**150**	34.0
	Lot 2	**92**	71-140	**150**	33.9
	Lot 3	**82**	64-121	**150**	33.8
	Lot 1-3	**92**	78-114	**150**	33.9

Table 24 Summary of LoD for pan-Sarbecovirus using WHO International Standard (NIBSC code: 20/146)

Viral Strain	Kit lot	95% Probit [IU/mL]	95% CI of Probit [IU/mL]	Hit rate ≥ 95% [IU/mL]	Mean Ct at ≥ 95% Hit rate
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	Lot 1	**62**	48-94	**75**	36.6
	Lot 2	**75**	54-128	**150**	35.6
	Lot 3	**79**	58-130	**75**	36.5
	Lot 1-3	**72**	60-92	**75**	36.5

Inclusivity

The inclusivity of **cobas**® SARS-CoV-2 Qualitative for the detection of SARS-CoV-2 was confirmed by testing nine SARS-CoV-2 strains, including six variant strains. The lowest target analyte at which all four tested replicates were positive are reported in Table 25.

Table 25 Summary of inclusivity

Strain	Catalog Number	Lot Number	Test Concentration with 100% Positivity
Hong Kong/VM20001061/2020	0810590CFHI	325659	1.06E+02 cp/mL
Italy-INMI1	0810589CFHI	325658	1.00E+02 cp/mL
USA-WA1/2020	0810587CFHI	325656	5.03E+01 cp/mL
UK (B.1.1.7)	0810614CFHI	326230	2.4E+01 cp/mL
Japan / Brazil (P.1)	NR-54982	70042875	1.9E+02 cp/mL
South Africa (B.1.351)	0810613CFHI	326229	2.4E+01 cp/mL
US NY (B.1.526)	NR-55359	70043342	1.9E+02 cp/mL
India (B.1.617.1)	NR-55486	70044706	2.5E+02 cp/mL
India (B.1.617.2)	NR-55611	70045238	7.0E+01 cp/mL

Precision

Within-laboratory precision was examined using a panel of SARS-CoV-2 (USA-WA1/2020, heat-inactivated) cultures diluted in simulated clinical matrix in universal transport media. Sources of variability were examined with a panel consisting of three concentration levels, using three lots of **cobas**® SARS-CoV-2 Qualitative reagents and three instruments over a course of 15 instrument days (2 runs/day x 3 instruments x 5 days/instrument) for a total of 30 runs. A description of the precision panel and the observed positivity rates are shown in Table 26. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation (CV) of the Ct values from tests performed on positive panel members (see Table 27) yielded overall CV percentage ranging from 1.1% to 2.2% for **cobas**® SARS-CoV-2 Qualitative.

Table 26 Summary of within laboratory precision

Target	Panel Member	Level (x LoD)	Positive Results	Total Results	Positivity %	Two-sided 95% CI Lower Bound	Two-sided 95% CI Upper Bound
Target 1 (SARS-CoV-2)	Weak positive	~0.3x	9	90	10%	5%	18%
	Low positive	~1.0x	82	90	91%	83%	96%
	Moderate positive	~3.0x	90	90	100%	96%	100%
Target 2 (pan-Sarbecovirus)	Weak positive	~0.3x	31	90	34%	25%	45%
	Low positive	~1.0x	84	90	93%	86%	97%
	Moderate positive	~3.0x	90	90	100%	96%	100%
N/A	Negative	Blank	0	90	0%	0%	4%

Table 27 Overall mean, standard deviation, and percent coefficient of variation for Ct values by positive panel member

Target	Level (x LoD)	Hit rate	Mean Ct	Instrument-to-Instrument		Lot-to-Lot		Day-to-Day		Run-to-Run		Within Run		Total	
				SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Target 1 (SARS-CoV-2)	~0.3x	10.0%	32.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.4	0.5	1.4
	~1.0x	91.1%	32.1	0.0	0.0	0.2	0.6	0.1	0.3	0.0	0.0	0.6	1.8	0.6	1.9
	~3.0x	100.0%	31.18	0.0	0.0	0.2	0.7	0.0	0.0	0.0	0.0	0.3	0.9	0.4	1.1
Target 2 (pan-Sarbecovirus)	~0.3x	34.4%	35.36	0.0	0.0	0.5	1.3	0.3	0.8	0.1	0.2	0.5	1.5	0.8	2.2
	~1.0x	93.3%	34.21	0.0	0.0	0.1	0.3	0.2	0.6	0.0	0.0	0.7	2	0.7	2.2
	~3.0x	100.0%	32.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.1	0.4	1.1

Analytical specificity / cross-reactivity

A panel of 48 viruses, bacteria, and fungi (including those commonly found in respiratory tract) were tested with **cobas®** SARS-CoV-2 Qualitative to assess analytical specificity. The organisms listed in Table 28 were spiked at concentrations of 1 x 10^5 units/mL for viruses and 1 x 10^6 units/mL for other organisms, unless otherwise noted.

Testing was performed with each potential interfering organism in the absence and presence of SARS-CoV-2 target (spiked at ~3x LoD). None of the organisms interfered with the test performance. Testing of SARS-CoV-1 generated an expected pan-Sarbecovirus positive result.

Table 28 Cross-reactivity test results

Microorganism	Concentration
Human coronavirus 229E	1.0E+05 $TCID_{50}$/mL
Human coronavirus OC43	1.0E+05 $TCID_{50}$/mL
Human coronavirus HKU1	1.0E+05 $TCID_{50}$/mL
Human coronavirus NL63	1.0E+05 $TCID_{50}$/mL
MERS coronavirus	1.0E+05 genomic equivalent/mL
SARS coronavirus	1.0E+05 PFU/mL
Adenovirus B (Type 34)	1.0E+05 $TCID_{50}$/mL
Bocavirus	1.0E+05 cp/mL
Cytomegalovirus	1.0E+05 $TCID_{50}$/mL
Epstein Barr virus	1.0E+05 cp/mL
Human Metapneumovirus (hMPV)	1.0E+05 $TCID_{50}$/mL
Measles virus	1.0E+05 $TCID_{50}$/mL
Mumps virus	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus Type 1	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus Type 2	1.0E+05 $TCID_{50}$/mL
Parainfluenza virus Type 3	1.0E+05 $TCID_{50}$/mL

Microorganism	Concentration
Parainfluenza virus Type 4	1.0E+05 $TCID_{50}$/mL
Influenza A (H1N1)	1.0E+05 $TCID_{50}$/mL
Influenza A virus (H1N1-2009, H1N3, H3N2)	1.0E+05 $TCID_{50}$/mL
Influenza B	1.0E+05 $TCID_{50}$/mL
Enterovirus E (Type 1)	1.0E+05 $TCID_{50}$/mL
Parechovirus	1.0E+05 $TCID_{50}$/mL
Respiratory syncytial virus	1.0E+05 PFU/mL
Rhinovirus	1.0E+05 $TCID_{50}$/mL
Candida albicans	1.0E+06 CFU/mL
Chlamydia pneumoniae	1.0E+06 $TCID_{50}$/mL
Corynebacterium diphtheriae	1.0E+06 CFU/mL
Escherichia coli	1.0E+06 CFU/mL
Haemophilus influenzae	1.0E+06 CFU/mL
Lactobacillus gasseri	1.0E+06 CFU/mL
Legionella pneumophila	1.0E+06 CFU/mL
Legionella jordanis (non-pneumophila)	1.0E+06 CFU/mL
Moraxella catarrhalis	1.0E+06 CFU/mL
Mycobacterium tuberculosis	1.0E+06 cells/mL
Neisseria elongata	1.0E+06 CFU/mL
Neisseria meningitidis	1.0E+06 CFU/mL
Pseudomonas aeruginosa	1.0E+06 CFU/mL
Pneumocystis jirovecii	1:20 of Patient Sample
Staphylococcus aureus	1.0E+06 CFU/mL
Staphylococcus epidermidis	1.0E+06 CFU/mL
Streptococcus pneumonia	1.0E+06 CFU/mL
Streptococcus pyrogenes	1.0E+06 CFU/mL
Streptococcus salivarius	1.0E+06 CFU/mL
Bordetella pertussis	1.0E+06 CFU/mL
Mycoplasma pneumoniae	1.0E+06 CFU/mL
Pooled Nasal Wash	1:20 of Patient Sample

Interference

The effect of exogenous substances potentially secreted into respiratory specimens was evaluated (Table 29). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence of SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results.

Table 29 List of exogenous substances tested for interference

Substance	Concentration
Oxymetazoline	0.011 mg/mL
Galphimia glauca, Luffa operculata, Sabadilla	0.023 mg/mL
Lidocaine and Phenylephrine	2.68 mg/mL
Budesonide	0.039 mg/mL
Phenol	0.47 mg/mL
Fluticasone propionate	166.67 µg/mL
Mupirocin	0.20 mg/mL
Zanamivir	0.0015 mg/mL
Oseltamivir	0.0073 mg/mL
Benzocaine and Menthol	5.00 mg/mL
Tobramycin	0.018 mg/mL
Petroleum Jelly	1% (w/v)
Nicotine	1% (w/v)
Camphor-synthetic eucalyptus oil and menthol ointment	1% (w/v)
0.65% NaCl, Phenylcarbinol, Benzalkonium chloride	1% (w/v)

Endogenous substances that may be present in respiratory specimens were tested for interference (Table 30). Each potentially interfering substance was tested at or above clinically relevant levels in negative simulated clinical matrix stabilized in universal transport media in absence and presence of SARS-CoV-2 target (spiked at ~3x LoD).

None of the substances interfered with the test performance by generating false-negative, false-positive or invalid results.

Table 30 List of endogenous substances tested for interference

Substance	Concentration
Human Genomic DNA	20 ng/µL
Mucus	One sputum swab/mL
Human Peripheral Blood Mononuclear Cells (PBMC)	1.0E+03 cells/µL
Human Whole Blood	1% (v/v)
Human Whole Blood	2% (v/v)
Human Whole Blood	5% (v/v)

Matrix equivalency – UTM-RT/UVT, cobas® PCR Media and 0.9% physiological saline

Equivalence between different collection media (UTM-RT/UVT, **cobas®** PCR Media, and saline) was evaluated. Equivalence between UTM-RT/UVT and **cobas®** PCR Media was evaluated the WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146). The WHO International Standard was used to formulate to a target concentration of approximately 2x LoD (low positive) and 4x LoD (moderate positive) into paired individual negative clinical samples, stabilized either in Universal Transport Media (UTM-RT/UVT) or, **cobas®** PCR Media (CPM).

Equivalence between UTM-RT/UVT and 0.9% physiological saline was evaluated using cultured virus (USA-WA1/2020 strain). The cultured virus was used to formulate to a target concentration of approximately 2X LoD (low positive) and 4X LoD (moderate positive) into paired individual negative clinical samples, stabilized either in Universal Transport Media (UTM-RT/UVT) or 0.9% physiological saline (NaCl).

At least 20 replicates per low positive sample and 10 replicates per moderate positive sample were tested for each collection media type. All replicates tested were positive for SARS-CoV-2 in all the three collection media types. UTM-RT/UVT, **cobas®** PCR Media, and 0.9% physiological saline are acceptable for use with **cobas®** SARS-CoV-2 Qualitative.

Whole system failure

The whole system failure rate was assessed by testing 100 specimens of simulated clinical matrix spiked with WHO International Standard for SARS-CoV-2 RNA (acid-heat inactivated England/02/2020 isolate, NIBSC code: 20/146) to a concentration of approximately 3x LoD. The results of this study determined that all replicates were valid and positive for SARS-CoV-2, resulting in a whole system failure rate of 0% with an upper one-sided 95% confidence interval of 2.95%.

Cross contamination

Studies were performed to evaluate potential cross contamination on the **cobas®** 6800/8800 Systems using **cobas®** SARS-CoV-2 Qualitative. Cross-contamination can cause false positive results. In this performance study, the sample-to-sample cross-contamination rate of **cobas®** SARS-CoV-2 Qualitative was 0.0% (0/239; with an upper one-sided 95% CI of 1.25%) in UTM samples and 0.6% (3/480; with a 95% CI from 0.1% to 1.8%) in Saliva samples when alternating high viral level positive and negative samples were tested over multiple runs. High viral level positive samples in the study were prepared to generate a Ct value that exceeds the 95th percentile of all positive samples observed via real world monitoring of **cobas®** SARS-CoV-2 Qualitative (>10 million results). The likelihood of encountering such specimens in the routine use of **cobas®** SARS-CoV-2 Qualitative is proportional to SARS-CoV-2 prevalence in the testing population. Therefore, the sample-to-sample cross-contamination rate for saliva samples in routine use of **cobas®** SARS-CoV-2 Qualitative will likely be less than 0.6% x 5% x SARS-CoV-2 prevalence (in percent) in the testing population. With an assumed prevalence of 10%, the estimated cross-contamination rate would be 0.6% x 5% x 10% = 0.003%.

Performance in sample pools

The performance of **cobas**® SARS-CoV-2 Qualitative when testing nasopharyngeal samples collected in UTM or UVT was evaluated using one **cobas**® 6800 System and one **cobas**® 8800 System. Thirty positive samples were tested individually and in pools of 6 containing 1 positive and 5 negative samples, and in pools of 3 containing 1 positive and 2 negative samples. Additionally, negative samples were tested individually, in 20 negative pools of 6, and in 20 negative pools of 2.

The 30 individual positive specimens had pan-Sarbecovirus Target 2 Ct values between 15.1 – 35.3, including a subset of 8 Low Positive samples (~27% of the samples) with Target 2 Ct values between 33.4 and 35.3. The Low Positive subset of samples targeted within 2-3 Ct (actual 1.1 – 3) of the mean Ct for Target 2 at the Limit of Detection.

The performance of testing sample pools of 6 and pools of 3 containing one positive sample each, compared to testing individual samples, is shown inTable 31 and Table 32, respectively. Positive and presumptive positive results (as defined in Table 17) were used for the Positive Percent Agreement (pools vs. individual) calculations, as all the constituent samples would require re-testing as separate individual samples. Results are summarized for all samples, and separately summarized for the subset of Low Positive samples, for each tested pool size.

Table 31 Reactivity in positive sample pools of 6

Samples in Pools of 6	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Positive Percent Agreement (pools vs, individual)
Positive (Including Low Positive)	0	0	30*	30	100% (30/30) (95% CI: 88.6 – 100%)
Low Positive	0	0	8*	8	100% (8/8) (95% CI: 67.6 – 100%)

*Note: One low positive sample was presumptive positive when tested in a pool of 6.

Table 32 Reactivity in positive sample pools of 3

Samples in Pools of 3	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Positive Percent Agreement (pools vs, individual)
Positive (Including Low Positive)	0	0	30	30	100% (30/30) (95% CI: 88.6 – 100%)
Low Positive	0	0	8	8	100% (8/8) (95% CI: 67.6 – 100%)

The performance of testing sample pools of 6 and pools of 2 containing only negative samples compared to testing individual samples, is shown in Table 33.

Table 33 Specificity in negative sample pools of 6 and pools of 2

Pool Size	Negative Pool Results	Invalid Pool Results	Positive or Presumptive Positive Pool Results	Total N valid Pool Results	Observed Negative Rate
Pools of 6	20	0	0	20	100% (20/20) (95% CI: 83.9 – 100%)
Pools of 2	20	0	0	20	100% (20/20) (95% CI: 83.9 – 100%)

Note: Some positive samples may not be detected when diluted and tested in pools. Performance estimations above may underestimate the loss of detection from testing in pools. Laboratories should also consider the assay's limit of detection when evaluating testing in pools (see **Warnings and precautions**).

Clinical performance evaluation

Performance with clinical specimens – swab specimen types

The performance of **cobas**® SARS-CoV-2 Qualitative was evaluated across three studies with archived or fresh, prospectively collected specimens. Combined, all three studies compared the performance of **cobas**® SARS-CoV-2 Qualitative across four external testing sites (one site in the EU, three in the US) using a common highly sensitive CE-IVD SARS-CoV-2 assay as the comparator method. The specimens in all studies were collected into VTM.

The first study consisted of archived nasopharyngeal swab (NPS) specimens from individuals with signs and symptoms of a respiratory infection evaluated at one external site. The second study consisted of one external site evaluating archived specimens from individuals without symptoms or other reasons to suspect COVID-19. The final study was a large multi-center study with three external testing sites evaluating prospectively collected fresh clinical specimens from individuals with signs and symptoms of a respiratory infection. Participants from 12 geographically distributed enrollment centers provided NPS and NS (nasal swab) specimens as part of a dual collection procedure where (a) the collection order was varied such that the first specimen collected will be ~50% NPS and ~50% NS, and (b) the collection method for NS specimens was also varied to yield ~50% self-collected and ~50% healthcare worker–collected.

Across the three studies, a total of 1,500 SARS-CoV-2 nasopharyngeal swab specimen results were evaluable and included in the data analysis. The accuracy (method correlation) of the **cobas**® SARS-CoV-2 Qualitative in comparison to a highly sensitive CE-IVD SARS-CoV-2 assay is shown in Table 34. Overall, the **cobas**® SARS-CoV-2 Qualitative Positive Percent Agreement (PPA) was 97.2% (140/144) and Negative Percent Agreement (NPA) was 99.9% (1,354/1,356).

Table 34 Summary of NPS clinical performance of **cobas**® SARS-CoV-2 Qualitative

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Nasopharyngeal	SARS-CoV-2	1500	97.2% (140/144)	93.1%	98.9%	99.9% (1,354/1,356)	99.5%	100%

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

Additionally, the aforementioned prospective multi-center evaluation study was designed to evaluate the performance of the **cobas**® SARS-CoV-2 Qualitative test using NPS and NS specimens from subjects suspected of respiratory infection. This study used a composite comparator method wherein laboratory sites used up to 3 highly-sensitive CE-IVD SARS-CoV-2 assays to determine the infective status by majority rule. The composite comparator result was defined as the concordant results from 2 comparator assays (test A and test B). In case of discordance between the initial 2 comparator assays, the sample was tested by a third assay (test C) and the result of that test determined the composite comparator status.

When compared with the composite comparator result, **cobas**® SARS-CoV-2 Qualitative yielded a Positive Percent Agreement (PPA) of 98.7% for NPS and 96.2% for NS specimens. The Negative Percent Agreement (NPA) was 99.7% for NPS and 100% for NS specimens. **cobas**® SARS-CoV-2 Qualitative also demonstrated similar performance when using self-collected and healthcare worker collected nasal swab specimens as shown in Table 35.

Table 35 Summary of NPS/NS clinical performance of **cobas**® SARS-CoV-2 Qualitative - prospective evaluation

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Nasopharyngeal*	SARS-CoV-2	938	98.7% (77/78)	93.1 %	99.8 %	99.7% (857/860)	99.0 %	99.9 %
Nasal Swab	SARS-CoV-2	941	96.2% (76/79)	89.4 %	98.7 %	100.0% (862/862)	99.6 %	100.0 %
Nasal Swab - Self Collected	SARS-CoV-2	481	100.0% (40/40)	91.2 %	100.0 %	100.0% (441/441)	99.1 %	100.0 %
Nasal Swab - HCW Collected	SARS-CoV-2	460	92.3% (36/39)	79.7 %	97.3 %	100.0% (421/421)	99.1 %	100.0 %

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit, HCW= healthcare worker.

* Nasopharyngeal specimen data from the prospective study are included in both Table 34 and Table 35. Test A of the SARS-CoV-2 composite comparator was the same method used as the single comparator in the summary analysis of all three studies.

Performance with clinical specimens – saliva specimen

The performance of **cobas**® SARS-CoV-2 Qualitative was evaluated with prospectively collected specimens. The study compared the performance of **cobas**® SARS-CoV-2 Qualitative at one external testing site within the EU against the paired nasopharyngeal swab result of **cobas**® SARS-CoV-2 Qualitative as the comparator. Nasopharyngeal specimens were collected into RT-UTM and saliva specimens were collected as raw saliva into a sterile device.

The study was evaluating prospectively collected clinical specimens from individuals with signs and symptoms of a respiratory infection, as well from individuals without signs and symptoms of a respiratory infection. Participants provided nasopharyngeal swab and saliva specimens as part of a dual collection procedure.

Paired specimens in a total of 652 subjects were evaluable and included in the data analysis, which included 298 (45.7%) who were symptomatic and 354 (54.3%) who were asymptomatic at the time of sample collection. The accuracy (method correlation) of the **cobas**® SARS-CoV-2 Qualitative using saliva in comparison to the **cobas**® SARS-CoV-2 Qualitative using nasopharyngeal swab specimen is shown in Table 36. Overall, the **cobas**® SARS-CoV-2 Qualitative Positive Percent Agreement (PPA) between the saliva and nasopharyngeal swab swab specimen types was 82.2% (120/146) and Negative Percent Agreement (NPA) was 97.2% (492/506).

Table 36 Summary of clinical performance of **cobas®** SARS-CoV-2 Qualitative using saliva in comparison to NPS

Specimen Type	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI	NPA	NPA LCL 95% Score CI	NPA UCL 95% Score CI
Saliva	SARS-CoV-2	652	82.2% (120/146)	75.2%	87.5%	97.2% (492/506)	95.4%	98.3%

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

The Positive Percent Agreement of the **cobas®** SARS-CoV-2 Qualitative using saliva in comparison to the **cobas®** SARS-CoV-2 Qualitative using nasopharyngeal swab specimen split into arbitrary viral level groups is shown in Table 37. The **cobas®** SARS-CoV-2 Qualitative Positive Percent Agreement (PPA) between the specimen types saliva and nasopharyngeal swab was 97.9.% (47/48) for NPS samples with a high viral level (Ct of target 1 (SARS-CoV-2) ≤ 23), 100.0% (50/50) for NPS samples with a moderate viral level (Ct of target 1 (SARS-CoV-2) > 23 to 30), and 47.9% (23/48) for NPS samples with a low viral level at and below the Limit of Detection of the NPS sample type (Ct of target 1 (SARS-CoV-2) > 30).

Table 37 Positive Percent Agreement **cobas®** SARS-CoV-2 Qualitative using saliva in comparison to the viral level detected in the paired NPS sample

Viral level based on Ct* of paired NPS specimen	Target	Total (N)	PPA	PPA LCL 95% Score CI	PPA UCL 95% Score CI
High (NPS Ct ≤ 23)	SARS-CoV-2	48	97.9% (47/48)	89.1%	99.6%
Moderate (NPS > Ct 23 to ≤ Ct 30)	SARS-CoV-2	50	100.0% (50/50)	92.9%	100.0%
Low (NPS Ct > 30, at and below LoD of NPS sample type)	SARS-CoV-2	48	47.9% (23/48)	34.5%	61.7%

CI= confidence interval, LCL= lower confidence limit, NPA= negative percent agreement, PPA= positive percent agreement, UCL= upper confidence limit.

* Ct of target 1 (SARS-CoV-2)

Testing of the 40 saliva specimens where results were discrepant between the paired nasopharyngeal swab and saliva specimen by an alternative highly sensitive CE-IVD NAT test resulted in a 100% agreement with the **cobas**® SARS-CoV-2 Qualitative saliva result. All 14 **cobas**® SARS-CoV-2 Qualitative NPS negative, but saliva positive results were confirmed as saliva positive by the alternative test, and all 26 **cobas**® SARS-CoV-2 Qualitative NPS positive, but saliva negative results were confirmed as saliva negative by the alternative test. This indicates that discrepant results when using saliva as a sample type are dependent on the differences between the two specimen types rather than on the assay performance.

Additionally, head-to-head comparison between saliva samples tested with the cobas® SARS-CoV-2 Qualitative and the Hologic Aptima™ SARS-CoV-2 tests was performed (Table 38). The two tests comparably detected SARS-CoV-2 RNA in saliva specimens with an overall Positive Percent Agreement (PPA) of 97.8% (131/134) and a Negative Percent Agreement (NPA) of 99.4% (514/517). The 95% confidence limits ranged from 93.6% to 99.2% for the PPA and from 98.3% to 99.8% for the NPA, respectively. All cobas-/Aptima+ saliva specimens generated negative results for the paired NPS specimens. For the cobas+/Aptima- saliva specimens, two of three results were positive for the paired NPS specimens. The third cobas+/Aptima- saliva specimen tested positive for target 2 only (pan-Sarbecovirus) with a late Ct value indicating a low SARS-CoV-2 RNA level near the limit of detection.

Table 38 Correlation between **cobas**® SARS-CoV-2 Qualitative and Aptima™ SARS-CoV-2 Test

Specimen Type	SARS-CoV-2				PPA [95% Score CI]	NPA [95% Score CI]
	Con +	Con -	cobas+ Aptima -	cobas - Aptima +		
Saliva	131	515	3	3	**97.8%** (131/134) [93.6–99.2%]	**99.4%** (515/518) [98.3–99.8%]

Con = Concordant; + = Positive; - = Negative, CI= confidence interval, NPA= negative percent agreement, PPA= positive percent agreement

Reproducibility

The reproducibility of **cobas**® SARS-CoV-2 Qualitative was evaluated across multiple factors that theoretically could affect reported results, including: reagent lot, testing site/instrument, day, and run. The evaluation was conducted at 3 testing sites, using 3 reagent lots, with a 4-member panel of positive and negative samples resulting in a total number of 216 tests per concentration (not including controls). The positive panel members contained SARS-CoV-2 viral culture material [WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)] at 3 different concentrations in universal transport medium (UTM) based simulated clinical matrix. Each site tested two reagent lots for 6 days. Two runs were performed each day and 3 replicates of each panel member were performed for each run. An overall SARS-CoV-2 positive result was determined by a positive detection in either or both of the SARS-CoV-2 or/and pan-Sarbecovirus channels. The evaluation results are summarized in Table 39.

The test results showed good lot-to-lot, instrument-to-instrument (site), day-to-day, and between batch variability for the ~0.3x LoD, ~1x LoD, and ~3x LoD panel members (Table 39). Regardless of viral targets and viral concentrations, most of the variability was within batches, ranging from 79.5% to 100%. Site-to-site variability ranged from 0.0% to 10.1%, and between-batch variability ranged from 0.0% to 16.0%.

Table 39 Overall mean estimate, standard deviations, and coefficients of variation (%) for cycle threshold values by viral target and expected viral concentration (positive panel members)

Viral Target	Panel Member Concentration	N*/N	Mean Ct**	Site SD	Site CV(%)	Lot SD	Lot CV(%)	Day SD	Day CV(%)	Batch SD	Batch CV(%)	Within Batch SD	Within Batch CV(%)	Total SD**	Total CV(%)***
SARS-CoV-2	~0.3x LoD	45/216	33.6	0.00	0.0	0.00	0.0	0.11	0.3	0.00	0.0	0.35	1.1	0.37	1.1
SARS-CoV-2	~1x LoD	196/216	33.2	0.00	0.0	0.09	0.3	0.00	0.0	0.17	0.5	0.37	1.1	0.42	1.3
SARS-CoV-2	~3x LoD	216/216	32.2	0.05	0.2	0.02	0.1	0.00	0.0	0.03	0.1	0.24	0.8	0.25	0.8
pan-Sarbecovirus	~0.3x LoD	158/216	36.5	0.18	0.5	0.00	0.0	0.00	0.0	0.00	0.0	0.71	2.0	0.74	2.0
pan-Sarbecovirus	~1x LoD	214/216	35.4	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.67	1.9	0.67	1.9
pan-Sarbecovirus	~3x LoD	216/216	34.1	0.11	0.3	0.05	0.2	0.00	0.0	0.00	0.0	0.32	0.9	0.34	1.0

Ct = cycle threshold; LoD = limit of detection; SD = standard deviation; CV(%) = percent coefficient of variation; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Note: SARS-CoV-2 is a dual target assay. Inactivated viral culture material was diluted to ~0.3/1/3x LoD based on the target 2 (SARS-CoV-2) LoD.

* n is the number of positive tests which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

** The mean and total standard deviation (SD) estimates were calculated from the PROC MIXED procedure.

*** Total CV(%) = (SD/Mean)*100.

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The system showed a 99.1% negative percent agreement with a 95% CI of 96.7 - 99.9%. Of the 216 valid tests, 2 tests were positive (1 each for SARS-CoV-2 and pan-Sarbecovirus). Post-amplification DNA sequencing confirmed the presence of an amplification product in 1 sample (pan-Sarbecovirus positive, Ct 36.7) and did not detect amplification product for either target in the other (SARS-CoV-2 positive, Ct 34.4). The Ct values and the curve analysis of the reactive negative panel member may suggest a low level of contamination during specimen handling.

System equivalency / system comparison

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

Additional information

Key test features

Sample type	Nasopharyngeal and oropharyngeal swab samples collected in the Copan UTM-RT System or the BD™ UVT System Nasal swab samples collected in the Copan UTM-RT System, the BD™ UVT System, the **cobas**® PCR Media, and 0.9% physiological saline Saliva samples
Minimum amount of sample required	Swab specimen types: 0.6 or 1.0 mL*,** Liquified Saliva: 1.2 mL
Sample processing volume	Swab specimen types: 0.4 mL Liquified Saliva: 0.85 mL

*Dead volume of 0.2 mL is identified for the **cobas omni** Secondary Tubes. Dead volume of 0.6 mL is identified for the **cobas**® PCR Media primary tubes. Other tubes compatible with **cobas**® 5800/6800/8800 Systems (consult User Assistance Guides) may have different dead volume and require more or less minimum volume.

**Additional volume is required if pooling.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 40 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <n> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and importer

Table 41 Manufacturer and importer

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

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EC REP Roche Diagnostics GmbH
Sandhofer Str. 116
68305 Mannheim
Germany

CE IVD

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1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.

2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 11/2021	First Publishing.
Doc Rev. 2.0 01/2022	The claim was extended to run additionally on the **cobas**® 5800 System, and with that all information required were added to the whole IFU. Please contact your local Roche Representative if you have any questions.

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress SARS-CoV-2

Instructions for Use
For Use Under an Emergency Use Authorization (EUA) Only

℞ only

REF XPRSARS-COV2-10

For Use with GeneXpert Dx or GeneXpert Infinity Systems

Trademark, Patents and Copyright Statements

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Xpert® Xpress SARS-CoV-2

For use under the Emergency Use Authorization (EUA) only.

1 Proprietary Name

Xpert® Xpress SARS-CoV-2

2 Common or Usual Name

Xpert Xpress SARS-CoV-2

3 Intended Use

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/ aspirate) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing of nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high or moderate complexity tests.

Testing of nasopharyngeal, nasal, or mid-turbinate swab specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing of these specimens is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

The Xpert Xpress SARS-CoV-2 test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

5 Principle of the Procedure

The Xpert Xpress SARS-CoV-2 test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.5 mL per cartridge
• Binding Reagent	1.5 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
Disposable Transfer Pipettes	**10-12 per kit**
CD	**1 per kit**
• Assay Definition File (ADF)	
• Instructions to import ADF into GeneXpert software	
Flyer	**1 per kit**
• Directions to locate the Product Insert on www.cepheid.com	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and postmortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

  For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

  For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

9 Materials Available but Not Provided

SeraCare AccuPlex™ Reference Material Kit, catalog number 0505-0126 (Order Code CEPHEID)

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- For emergency use only.
- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other virus or pathogens.
- This product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb- 3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Positive results are indicative of presence of SARS-CoV-2-RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Performance characteristics of this test have only been established with nasopharyngeal swab specimens. The performance of this assay with other specimen types or samples has not been evaluated.

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[3] and the Clinical and Laboratory Standards Institute.[4]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress SARS-CoV-2 cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.

- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Xpress SARS-CoV-2 cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

11 Chemical Hazards[5,6]

- Signal Word: WARNING
- **UN GHS Hazard Statements**
  - Harmful if swallowed.
  - May be harmful in contact with skin.
  - Causes eye irritation.
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure, Section 12.2 for oropharyngeal swab collection procedure, Section 12.3 for nasal swab collection procedure, Section 12.4 for mid-turbinate swab collection procedure, and Section 12.5 for nasal wash/aspirate procedure. Nasopharyngeal, nasal, and mid-turbinate swabs and nasal wash/aspirate specimens can be stored at room temperature (15-30 °C) for up to 8 hours and refrigerated (2-8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems. For oropharyngeal swab specimen transport and storage requirements and additional information, refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) using the link provided below.

https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

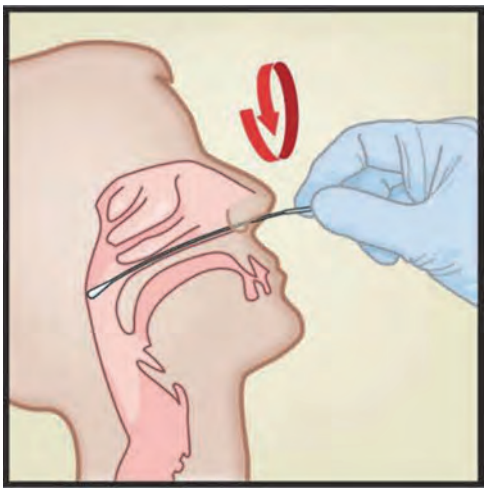


Figure 1. Nasopharyngeal Swab Collection

12.2 Oropharyngeal Swab Collection Procedure

1. Swab the posterior pharynx, tonsils, and other inflamed areas. Avoid touching the tongue, cheeks, and teeth with the swab when collecting specimens.
2. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.3 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.4 Mid-Turbinate Swab Collection Procedure

1. Insert the mid-turbinate swab into either nostril, passing it into the mid-turbinate area (see Figure 4). Rotate swab by firmly brushing against the mid-turbinate area several times.
2. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

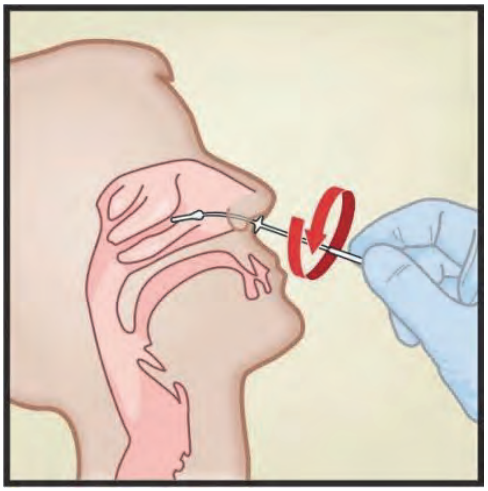


Figure 4. Mid-turbinate Swab Specimen Collection

12.5 Nasal Wash/Aspirate Procedure

Using a clean transfer pipette, transfer 600 µL of the sample into the tube containing 3 mL of viral transport medium or 3 mL of saline and then cap the tube.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 5).

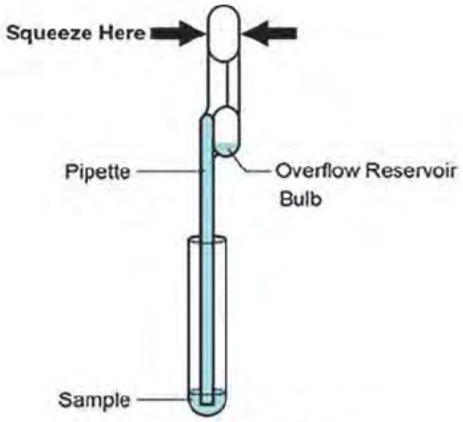


Figure 5. Transfer Pipette

7. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (see Figure 5). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 6. Dispose of the used pipette.

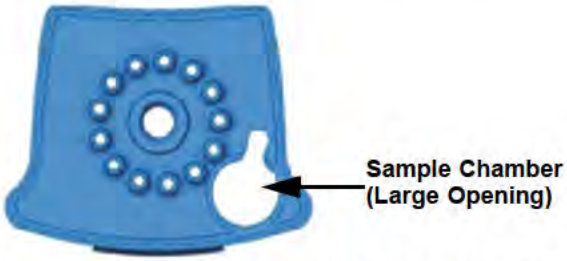


Figure 6. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress SARS-CoV-2 test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 6.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress SARS-CoV-2 Assay Definition File is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - **GeneXpert Dx:**
     If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

     or
   - **GeneXpert Infinity System:**
     If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.

Note If the barcode on the Xpert Xpress SARS-CoV-2 cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

For the GeneXpert Dx Instrument

A. Locate the module with the blinking green light, open the instrument module door and load the cartridge.

B. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

C. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

or

For the GeneXpert Infinity System

A. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.

B. When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 Internal Controls

CONTROL

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) - Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) - Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check - PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation (Continued)

Result	Interpretation
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the target nucleic acid reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

17 Retests

17.1 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- A **PRESUMPTIVE POS** result indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Only one of the SARS-CoV-2 nucleic acid target was detected (E gene) while the other SARS-CoV-2 nucleic acid target (N2 gene) was not detected.
- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR)** or a **PRESUMPTIVE POS** result, use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2 cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

18 Limitations

- Performance of the Xpert Xpress SARS-CoV-2 test has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress SARS-CoV-2 test with other specimen types has not been assessed and performance characteristics are unknown.
- Oropharyngeal, nasal swabs and mid-turbinate swabs (self-collected under supervision of or collected by a healthcare provider) as well as nasal wash/aspirate are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS- CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.

19 Conditions of Authorization for Laboratories

The Cepheid Xpert Xpress SARS-CoV-2 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:
https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-
However, to assist clinical laboratories and/or Point of Care Settings using the Xpert Xpress SARS-CoV-2 (referred to in the Letter of Authorization as “Your Product”), the relevant Conditions of Authorization are listed below.

- Authorized laboratories[a] using your product must include with test result reports all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories using your product must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA Reporting@fda.hhs.gov) and Cepheid (+1 888 838 3222 or techsupport@cepheid.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- You, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

a. The letter of authorization refers to "authorized laboratories" as follows: "Testing of nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx and GeneXpert Infinity Systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high or moderate complexity tests. Testing of nasopharyngeal, nasal, or mid-turbinate swab specimens using the Xpert Xpress SARS- CoV-2 test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is limited to laboratories certified under CLIA that meet requirements to perform high, moderate, or waived complexity tests. Testing of these specimens is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation."

20 Performance Characteristics

20.1 Clinical Evaluation

The performance of the Xpert Xpress SARS-CoV-2 test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium. A total of 45 SARS-CoV-2 positive and 45 SARS-CoV-2 negative NP swab specimens were tested with Xpert Xpress SARS-CoV-2 in a randomized and blinded fashion.

All the 45 SARS-CoV-2 positive specimens and 30 of the 45 SARS-CoV-2 negative specimens were collected during COVID-19 pandemic in the US and had previously been characterized as positive or negative for SARS-CoV-2 by an EUA RT-PCR test. Fifteen of the 45 SARS-CoV-2 negative NP swab specimens were collected before December 2019 and are expected to be negative for SARS-CoV-2.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress SARS-CoV-2 test relative to the expected results. Results of these 90 archived clinical NP swab specimens are shown in Table 3. The PPA was 97.8% (95% CI: 88.4% - 99.6%) and the NPA was 95.6% (95% CI: 85.2% - 98.8%).

Table 3. Xpert Xpress SARS-CoV-2 Performance Results

		Expected Results		
		Positive	**Negative**	**Total**
Xpert Xpress SARS-CoV-2	**Positive**	44[a]	2[b]	46
	Negative	1	43	44
	Total	45	45	90
PPA		97.8% (95% CI: 88.4% - 99.6%)		
NPA		95.6% (95% CI: 85.2% - 98.8%)		

a. One specimen was reported as "SARS-CoV-2 Presumptive Pos" in initial testing and yielded a "SARS-CoV-2 Positive" test result upon retesting.
b. The two false positive specimens were collected during the COVID-19 pandemic.

21 Analytical Performance

21.1 Analytical Sensitivity (Limit of Detection) – Live SARS-CoV-2 Virus

Studies were performed to determine the analytical limit of detection (LoD) of the Xpert Xpress SARS-CoV-2. The LoD of Xpert Xpress SARS-CoV-2 was established using one lot of reagent and limiting dilutions of live SARS-CoV-2 virus (USA_WA1/2020 strain) prepared in viral transport medium and NP swab clinical matrix. The concentration level with observed hit rates greater than or equal to 95% in the LoD determination study were 0.0050 and 0.0200 PFU/mL for the N2 target and E target, respectively (Table 4).

Verification of the estimated LoD claim was performed on one reagent lot in replicates of 20 prepared in pooled NP swab clinical matrix. The LoD is the lowest concentration (reported as PFU/mL) of live SARS-CoV-2 virus samples that can be reproducibly distinguished from negative samples ≥ 95% of the time with 95% confidence. The claimed LoD is 0.0200 PFU/mL (Table 4).

Table 4. LoD Determination using USA-WA1/2020 Strain

Strain	Concentration (PFU/mL)	Total Valid Results	Hit Rate (%)	Hit Rate (%)	Mean Ct	Mean Ct
			N2 Target	E Target	N2 Target	E Target
SARS-CoV-2 virus (USA_WA1/2020)	0.0200	20	100	95.0	38.3	36.4
	0.0050	22	95.5	68.2	40.5	39.1
	0.0025	22	90.9	36.4	41.5	39.6
	0.0010	22	50.0	18.2	42.0	42.0
	0.0005	22	45.5	18.2	41.7	41.5
	0.0003	22	18.2	4.5	42.1	44.9
	0.0001	22	9.1	0	42.9	N/A
	0	0	0	0	N/A	N/A

21.2 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress SARS-CoV-2 was evaluated using in silico analysis of the assay primers and probes in relation to 110,206 SARS-CoV-2 sequences available (as of October 21, 2020) in the GISAID gene database for two targets, E and N2.

For the E target, 214 matching sequences were excluded due to ambiguity codes, which reduced the total to 109,992 sequences. Xpert Xpress SARS-CoV-2 had 99.14% match to the sequences with the exception of 926 sequences that had a single mismatch and 21 sequences with additional mismatches. The 21 sequences with additional mismatches were:

- One sequence contained one mismatch in the forward primer region and a second mismatch between oligos;
- One sequence contained one mismatch in the probe binding region and a second mismatch between oligos;
- One sequence contained two mismatches in the probe binding region,
- One sequence contained a 3-nucleotide deletion and multiple mismatches at the 3'-end of the probe region;
- Two sequences contained two mismatches in the forward primer region;
- Three sequences contained a 6-nucleotide deletion in the probe binding region with multiple mismatches at the 3' end of the amplicon; and
- Twelve sequences contained a 'AA' dinucleotide but this lies between the oligonucleotides used in the assay.

None of these mismatches are predicted to have a negative impact on the performance of the assay.

For the N2 target, 211 matching sequences were excluded due to ambiguity codes, which reduced the total to 109,995 sequences. Xpert Xpress SARS-CoV-2 had 97.29% match to the sequences with the exception of 2,919 sequences that had a single mismatch and sixty- three sequences with two or more mismatches. The 63 sequences with additional mismatches were:

- One sequence contained two mismatches in the probe binding region and a third mismatch between oligos;
- Two sequences contained one mismatch in the forward primer region and a second mismatch in the reverse primer region;
- Six sequences contained one mismatch in the forward primer region and a second mismatch between oligos;
- Twenty-seven sequences contained one mismatch in the probe binding region and a second mismatch in the reverse primer region; and
- Twenty-seven sequences contained one mismatch in the forward primer region and a second mismatch in the probe binding region.

None of these mismatches are predicted to have a negative impact on the performance of the assay.

21.3 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 5 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2 test individually to the sequences downloaded from the GISAID database (as of March 13, 2020). E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 5 is expected based on the *in silico* analysis.

Table 5. Xpert Xpress SARS-CoV-2 Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-pneumophila
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongate and meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Staphylococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

21.4 FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The samples were tested using the GeneXpert Dx System. The results are summarized in Table 6.

Table 6. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD (NDU/mL)	Cross-Reactivity
SARS-CoV-2	NP Swab	$5.4x10^3$	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

22 References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.
2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.
3. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). http://www.cdc.gov/biosafety/publications/
4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline.* Document M29 (refer to latest edition).
5. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).
6. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

23 Cepheid Headquarters Locations

Corporate Headquarters	European Headquarters
Cepheid 904 Caribbean Drive Sunnyvale, CA 94089 USA	Cepheid Europe SAS Vira Solelh 81470 Maurens-Scopont France
Telephone: +1 408 541 4191	Telephone: +33 563 825 300
Fax: +1 408 541 4192	Fax: +33 563 825 301
www.cepheid.com	www.cepheidinternational.com

24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag number

Region	Telephone	Email
US	+1 888 838 3222	techsupport@cepheid.com
France	+33 563 825 319	support@cepheideurope.com
Australia New Zealand	+1800 130 821 +0800 001 028	techsupportANZ@cepheid.com

Contact information for all Cepheid Technical Support offices is available on our website:
www.cepheid.com/en_US/support/contact-us

25 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not re-use
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
	Country of manufacture
	Contains sufficient for <n> tests
CONTROL	Control
	Expiration date
	Temperature limitation
	Biological risks
Rx only	For prescription use only

Cepheid
904 Caribbean Drive
Sunnyvale, CA 94089 USA
Phone: +1 408 541 4191
Fax: +1 408 541 4192

IVD

For use under Emergency Use Authorization (EUA) Only

For Professional Use Only

Allplex™ SARS-CoV-2 Assay

(Cat. No. RV10248X)

Multiplex real-time one-step RT-PCR system for detection of SARS-CoV-2 from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, sputum, saliva, and combo-swab (nasal swab + oral swab).

For use with

1. Microlab NIMBUS IVD and Microlab STARlet IVD

2. Seegene NIMBUS and Seegene STARlet

For use with

1. CFX96™ Real-time PCR System (CFX Manager™ Software-IVD v1.6)

2. CFX96™ Dx System (CFX Manager™ Dx Software v3.1)

3. Applied Biosystems™ 7500 (SDS software v2.0.5)

CE

Σ 100

IVD — **For *in vitro* diagnostic use only**

Seegene Inc.,
Taewon Bldg., 91 Ogeum-ro, Songpa-gu, Seoul, Republic of Korea 05548

EC REP — **Medical Technology Promedt Consulting GmbH**
Altenhofstrasse 80, D-66386 St.Ingbert, Germany

Not available in the U.S.

TABLE OF CONTENTS

NOTICES

- For *in vitro* diagnostic use only.
- The Allplex™ SARS-CoV-2 Assay should be performed by qualified, trained personnel.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- **This product is only for use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, and Seegene STARlet in maximum 5 separate runs.**
- **This test has been validated for the following specimen types: nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, sputum, saliva, and combo-swab (nasal swab + oral swab).** This test has not been validated for any other types of specimens.
- **Store RNA samples at ≤ -20°C until use and keep on ice during use.**
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.
- Workflow in a laboratory should proceed in an unidirectional manner.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink, or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use the product after its expiry date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive controls. To prevent contamination of reagents, the use of filter-tips is recommended.
- Use separated and segregated working areas for each experiment.

- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separately from the kit's reagents.
- Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components including product residuals and packaging can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiry date is 13 months from the date of manufacture at ≤ -20°C. Please refer to label for the expiry date.
- Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, respectively although the manufacturers are different from each other. Since there are no hardware changes on the devices, the test results are the same for those.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" has been changed to "CFX96™ Dx system". Since there are no hardware changes on the systems, it will be expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD v1.6". The upgraded software includes enhancements to the "Run" menu. These enhancements do not impact the results of data analysis; therefore, the results will be the same.
- This kit is a qualitative *in vitro* test for the single or multiple detection of 4 types of gene (E gene, RdRP gene, S gene, and N gene).

INTENDED USE

Allplex™ SARS-CoV-2 Assay is *in vitro* diagnostic medical device designed for qualitative detection of SARS-CoV-2 with real-time reverse transcription PCR from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, sputum, saliva, and combo-swab (nasal swab + oral swab).

PRINCIPLES AND PROCEDURE OVERVIEW

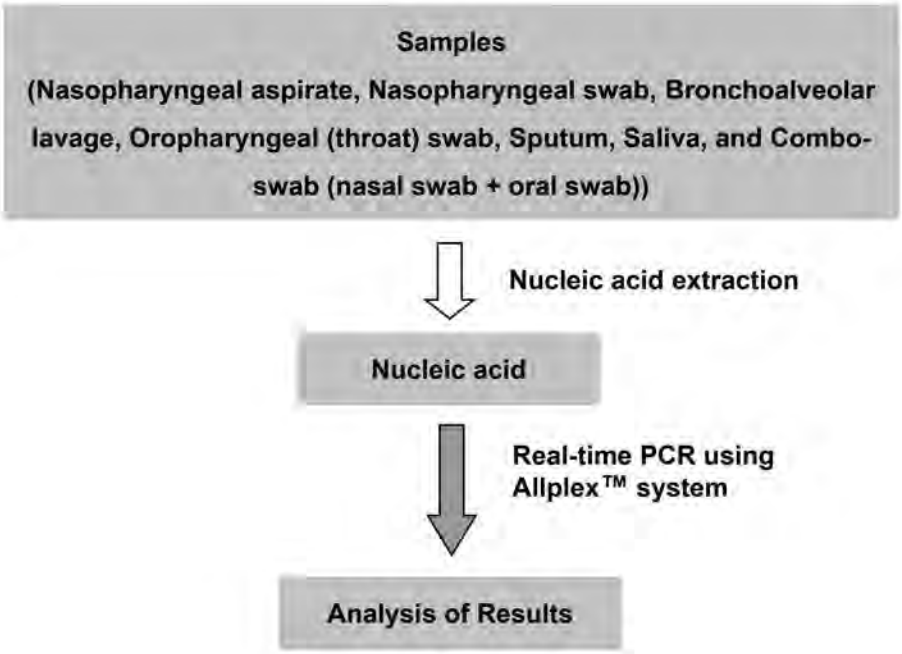
1. Principles

Allplex™ SARS-CoV-2 Assay is a multiplex real-time RT-PCR assay that enables simultaneous amplification and detection of target nucleic acids of E gene, RdRP gene, S gene, and N gene with Internal Control (IC). The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

An exogenous gene is used as Internal Control (IC) to monitor the whole process of nucleic acid extraction and to check for any possible PCR inhibition.

To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ SARS-CoV-2 Assay. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.

2. Procedure Overview



< Allplex™ SARS-CoV-2 Assay procedure overview >

BACKGROUND INFORMATION

1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known by the provisional name 2019 novel coronavirus (2019-nCoV), is the cause of the respiratory coronavirus disease 2019 (COVID-19). Taxonomically, it is a strain of the Severe acute respiratory syndrome-related coronavirus (SARSr-CoV), a positive-sense single-stranded RNA virus. It is contagious in humans, and the World Health Organization (WHO) has designated the ongoing pandemic of COVID-19 a Public Health Emergency of International Concern.

SARS-CoV-2 is believed to have zoonotic origins. It has close genetic similarity to bat coronaviruses, suggesting it emerged from a bat-borne virus. An intermediate animal reservoir such as a pangolin is also thought to be involved in its introduction to humans. Chinese scientists first isolated SARS-CoV-2 7 January 2020 from patients in Wuhan, China who had had pneumonia of unknown cause in December 2019.

REAGENTS

The reagents contained in one kit are sufficient for 100 reactions.

Order information (REF Cat. No. RV10248X)

Allplex™ SARS-CoV-2 Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	SARS2 MOM	500 µL	Oligo Mix: - Amplification and detection reagent
PREMIX	EM8	500 µL	- RTase - DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	SARS2 PC	50 µL	Positive Control (PC): - Mixture of pathogen and IC clones
CONTROL IC	RP-V IC 2	1,000 µL	Exogenous Internal Control (IC)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

STORAGE AND HANDLING

All components of Allplex™ SARS-CoV-2 Assay should be stored at ≤ -20°C. All components are stable under recommended storage conditions until the expiry date stated on the label. The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and Sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Nucleic acid extraction kit (see Nucleic Acid Extraction)
- Clean bench
- Ice Maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Applied Biosystems™ 7500 (Thermo Fisher Scientific)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)*
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)*
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad)*
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad)*
- Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)* §
- PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)* §
- EU 0.1ml 8-tube strip, LP, W, Extra Robust (Cat. No. B72719, BIOplastics)*
- EU Optical Wide area 8-Cap Strip (Cat. No. B57801, BIOplastics)*
- 96 x 0.1ml Plate, LP, W, FULL, 96 well plate (Cat. No. B70679, BIOplastics)*
- Opti-Seal Optical Sealing Sheet (Cat. No. 157300, BIOplastics)*
- MicroAmp® Optical 96-Well Reaction Plate (Cat. No. N8010560, Thermo Fisher Scientific)**
- MicroAmp™ Optical 96-Well Reaction Plate with Barcode (Cat. No. 4306737, Thermo Fisher Scientific)**
- Optical Adhesive Covers (Cat No. 4360954, Thermo Fisher Scientific)**
- MicroAmp™ Optical 8-Tube Strip, 0.2 mL (Cat No. 4316567, Thermo Fisher Scientific)**
- MicroAmp™ Optical 8-Cap Strips (Cat No. 4323032, Thermo Fisher Scientific)**

* Products for CFX96™ Real-time PCR Detection and CFX96™ Dx System

** Products for Applied Biosystems™ 7500

§ Make sure to use the heat seal and the plate sealer listed above together.

PROTOCOL

1. Specimen Collection, Storage, and Transport

Note: All samples should be treated as potentially infectious materials. Only permitted are those sample materials, which are collected, transported and stored by attending strictly to the following rules and instructions.

Note: To ensure high quality of samples, samples should be transported as fast as possible at indicated temperature.

A. Specimen Collection

Nasopharyngeal aspirate, Nasopharyngeal swab, Bronchoalveolar lavage, Oropharyngeal (throat) swab

- Nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab specimens are routinely examined for common respiratory pathogens.
- Obtaining respiratory specimens may be difficult in some patients. In such cases, nasopharyngeal swabs may be collected simply and efficiently using new nylon flocked swabs (COPAN, Italy) and Universal Transport Medium (UTM).

Manufacturer	Specimen collection device	Cat. No.
COPAN	ESwab	482CE
COPAN	ENAT PM 2ML PERNASAL APPLICATOR**	606CS01P*
COPAN	UTM with Flocked Swabs	360C / 305C
DIAGNOSTIC HYBRIDS	UTM with Flexible Minitip Flocked Swab	403C / 406C
COPAN	MSwab® kit	6E012N / 6E013N
COPAN	MSwab® bulk	6E011N
Noble Biosciences	CTM (Clinical Virus Transport Medium)	UTNFS-3B-2-N1P / UTNFS-3B-2
SG Medical	GeneTM Set (GTS2)**	T5001
SG Medical	GeneTM Set (GTS1)**	T5002
SG Medical	ALLTM Set (ATS 1)	T5004
SG Medical	ALLTM Set (ATS 2)	T5003
SG Medical	ALLTM Medium	T5103

*Please use catalog numbers shown above to purchase products from Seegene Inc.

** ENAT PM 2ML PERNASAL APPLICATOR, GeneTM Set (GTS2) and GeneTM Set (GTS1) are not applicable to extraction-free method.

Sputum

- Give clear instructions to patients when collecting sputum specimens. Patients must either collect samples outside in the open air or away from other people. Patients should not collect samples in confined spaces such as toilets.
- Rinse mouth with water before collecting sputum. The patient should cough deeply and expectorate sputum directly into the container.
- A sputum sample must have a volume of 3~5 mL.

Saliva

- Do not eat, drink, smoke, brush teeth, or chew gum for 30 min before sample collection.
- Wash your hands thoroughly for 30 sec.
- Remove the cap of the sterilized empty tube.
  Note: Do not touch the inside of the cap and the inside of the sterilized tube.
- Gently expel saliva into the sterilized tube until 1~2 mL has been collected. Be very careful not to splash or aerosols.
  Note: If saliva gets on the outside of the tube, wipe it with an alcohol cotton pad.
- Screw the cap of the specimen tube tightly.

Combo-swab (nasal swab + oral swab)

- Give instruction to patients no to eat, drink, smoke, brush teeth, or chew gum within 1 hour before collecting combo-swab specimens.

a. Oral swab

- Before collecting the specimen, take a deep breath (while wearing a mask) and cough 3 to 5 times.
- Rub the collection tip of the swab over the inside of both cheeks, above and below the tongue, on both outer (gums), and on the hard palate for total of 20 seconds.
- Place swab, tip first, into the transport tube provided. Break the swab shaft at the scoreline and then close the lid.

b. Nasal swab

- Insert the entire collection tip of the swab approximately 3 to 4 cm inside the nostril.
- Firmly sample the nasal wall by rotating the swab in a circular path against the nasal wall for 5~10 seconds.
- Repeat in the other nostril using the same swab.
- Place swab, tip first, into the oral swab-containing transport tube provided. Break the swab shaft at the scoreline and then close the lid.

Manufacturer	Specimen collection device	Cat. No.
SG Medical	SELTM Set (STS2)*	T5021

* SELTM Set (STS2) consists of nasal swab, oral swab, and ALLTM Medium. Also, it is not applicable to extraction-free method.

B. Specimen Storage & Transport

Specimen	Storage & Transport		Note
	Temp.	Duration*	
Nasopharyngeal aspirate	2~8°C	3 days	- Performance may be affected by prolonged storage of specimens. - Specimens should also adhere to local and national instructions for transport of pathogenic materials.
Nasopharyngeal swab			
Bronchoalveolar lavage			
Oropharyngeal (throat) swab			
Sputum			
Combo-swab (nasal swab + oral swab)			
Saliva	2~8°C	4 days	
	15~25°C	2 days	

* Duration: The time period from the specimen collection to the final test (includes transport and storage of specimens prior to tests).

2. Nucleic Acid Extraction

[Extraction methods in different specimens]

Note: Please use the automated extraction system according to the specimen shown in the following table.

Specimen	Automated Extraction System							Extraction-free§
	Microlab NIMBUS IVD / STARlet IVD		Seegene NIMBUS / STARlet		KingFisher Flex*	MagNA Pure 96*	Maelstrom™ 9600**	
	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit ***	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit ***				
Nasopharyngeal aspirate	O	X	O	X	O	O	X	X
Nasopharyngeal swab	O	O	O	O	O	O	O	O
Bronchoalveolar lavage	O	X	O	X	X	X	X	X
Oropharyngeal (throat) swab	O	O	O	O	O	O	O	O
Sputum	O	X	O	X	O	O	O	X
Saliva§§	O	X	O	X	X	X	X	O
Combo-swab (nasal swab + oral swab)	O	O	O	O	O	O	O	X

* Bronchoalveolar lavage and saliva have not been validated with KingFisher Flex and MagNA Pure 96.

** Nasopharyngeal aspirate, bronchoalveolar lavage, and saliva have not been validated with Maelstrom™ 9600.

*** Nasopharyngeal aspirate, bronchoalveolar lavage, sputum, and saliva have not been validated with Viral DNA/RNA 200 C Kit.

§ Swab and saliva specimens are validated with extraction-free method. However, swab specimens using ENAT PM 2ML PERNASAL APPLICATOR, GeneTM Set (GTS2) and GeneTM Set (GTS1) are not applicable to extraction-free method.

§§ Saliva has been validated with Universal Cartridge Kit and extraction-free method.

2-1. Standard Extraction

A. Pre-treatment of specimen

Sputum

- Add 2 volumes of 1X PBS or saline solution to the 1 volume specimen in the 15 mL conical tube and vortex thoroughly to disperse the sample.
- Transfer recommended volume (See Recommended Vol. of 2-C) of sample to a new tube.
- Follow the extraction kit protocol.

Note: In case of the specimens without viscosity, pre- treatment step is NOT required.

Saliva

- Add 1 volume of 1X PBS to the 1 volume specimen in the sterilized tube and vortex thoroughly to disperse the sample.
- Transfer recommended volume (See Recommended Vol. of 2-C) of sample to a new tube.
- Follow the extraction kit protocol.

B. Internal Control

Note: IC, included in the kit, allows the user not only to verify the nucleic acid extraction procedure, but also to identify any PCR inhibition.

- RP-V IC 2 tube must be loaded on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet before nucleic acid extraction.
- When using KingFisher Flex, MagNA Pure 96 or Maelstrom™ 9600, 10 μL of RP-V IC 2 must be added to each specimen before nucleic acid extraction.

C. Automated Nucleic Acid Extraction System

Note: Please use the recommended volumes of specimen and elution as indicated below. For other matters, refer to the manufacturer's manual.

C-1. Microlab NIMBUS IVD

Note: See **Microlab NIMBUS IVD** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab NIMBUS IVD	Hamilton	65415-02*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Please use catalog numbers shown above to purchase products from Seegene Inc.
** Nasopharyngeal aspirate, bronchoalveolar lavage, sputum and saliva have not been validated.

C-2. Microlab STARlet IVD

Note: See **Microlab STARlet IVD** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab STARlet IVD	Hamilton	173000-075*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Please use catalog numbers shown above to purchase products from Seegene Inc.
** Nasopharyngeal aspirate, bronchoalveolar lavage, sputum and saliva have not been validated.

C-3. Seegene NIMBUS

Note: See **Seegene NIMBUS** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene NIMBUS	Seegene	65415-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Nasopharyngeal aspirate, bronchoalveolar lavage, sputum and saliva have not been validated.

C-4. Seegene STARlet

Note: See **Seegene STARlet** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene STARlet	Seegene	67930-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Nasopharyngeal aspirate, bronchoalveolar lavage, sputum and saliva have not been validated.

C-5. KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head

Note: See **KingFisher Flex** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head	Thermo Fisher Scientific	5400630	-
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher Scientific	A42352	Specimen: 200 µL Elution: 80 µL

Bronchoalveolar lavage and saliva have not been validated with KingFisher Flex.

C-6. MagNA Pure 96

Note: See **MagNA Pure 96** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
MagNA Pure 96	Roche Diagnostics	06541089001	-
MagNA Pure 96 DNA and Viral NA Small Volume Kit	Roche Diagnostics	06543588001	Specimen: 200 µL Elution: 100 µL

Bronchoalveolar lavage and saliva have not been validated with MagNA Pure 96.

C-7. Maelstrom™ 9600

- Proceed the extraction process using **'665-Rapid' protocol**

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Maelstrom™ 9600	Taiwan Advanced Nanotech Inc.	M9600	-
TANBead® Nucleic Acid Extraction Kit OptiPure Viral Auto Tube	Taiwan Advanced Nanotech Inc.	W665S66	Specimen: 300 μL Elution: 80 μL
TANBead® Nucleic Acid Extraction Kit OptiPure Viral Auto Plate	Taiwan Advanced Nanotech Inc.	W665A46	Specimen: 300 μL Elution: 80 μL
TANBead® Nucleic Acid Extraction Kit OptiPure Viral Bulk Plate	Taiwan Advanced Nanotech Inc.	W665A10	Specimen: 300 μL Elution: 80 μL

Nasopharyngeal aspirate, bronchoalveolar lavage, and saliva have not been validated with Maelstrom™ 9600.

2-2. Extraction-free

Note: RP-V IC 2 must be added to Mastermix during preparation for PCR. See Preparation for Real-time One-step RT-PCR (page 16) for more information.

A. Extraction-free for Swab

1) Aliquot 45 μL of Nuclease-free water into PCR tubes.
2) Add 15 μL of each sample into the tube containing aliquot of the Nuclease-free water.
3) Close the cap, quick vortex and briefly centrifuge.
4) Incubate at 98℃ for 3 min*.
5) Chill at 4℃ for 5 min*.

***Note:** It is recommended to perform steps 4)~5) on PCR instrument with PCR tube attached individual caps.

Note: If you need retesting, start with the original sample not the lysate.

B. Extraction-free for Saliva

1) Aliquot 30 μL of 1xTE buffer* into PCR tubes.
   *1xTE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0
2) Add 10 μL of each saliva sample into PCR tube containing aliquot of 1xTE buffer.
   Note: If saliva sample is viscous, add 3 volume of 1xTE buffer into 1 volume of saliva and vortex, thoroughly. Then, add 40 μL of the saliva & 1xTE buffer mixture into PCR tube.
3) Add 5 μL of Proteinase K into PCR tube containing the saliva and 1xTE buffer mixture.
   The final concentration of Proteinase K should be 2.22 mg/mL in total 45 μL.
4) Close the cap, quick vortex and briefly centrifuge.
5) Incubate at 98℃ for 20 min*.
6) Chill at 4℃ for 5 min*.

***Note:** It is recommended to perform steps 5)~6) on PCR instrument with PCR tube attached individual caps.

Note: If you need retesting, start with the original sample not the lysate.

3. Preparation for Real-time One-step RT-PCR

Note: Correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).

Note: Aerosol resistant filter tips and tight gloves must be used when preparing One-step RT-PCR reactions. Use extreme care to prevent cross-contamination.

Note: Completely thaw all reagents on ice.

Note: Briefly centrifuge reagent tubes to collect residual drops inside of the cap.

Note: The steps A~D are automatically processed on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet. Refer to each operation manual.

A. Prepare the Reaction Mastermix

A-1 Standard extraction

5 μL	SARS2 MOM
5 μL	EM8
5 μL	RNase-free Water
15 μL	Total volume of Mastermix

A-2 Extraction-free

5 μL	SARS2 MOM
5 μL	EM8
4 μL	RNase-free Water
1 μL	RP-V IC 2
15 μL	Total volume of Mastermix

Note: Calculate the total amount of each reagent needed based on the number of reactions including samples and controls.

B. Mix by quick vortexing, and briefly centrifuge.

C. Aliquot 15 μL of Reaction Mastermix into PCR tubes.

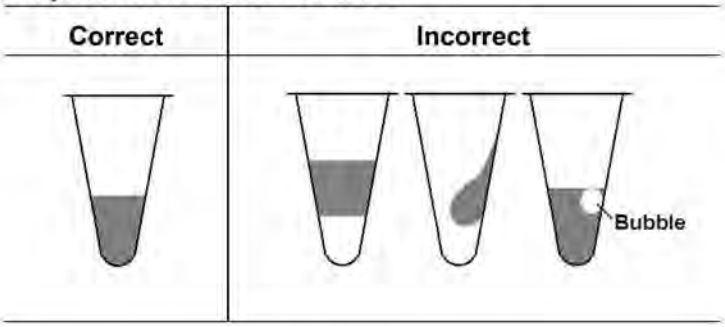
D. Add 5 μL of each sample's nucleic acids into the tube containing Reaction Mastermix.

15 μL	Reaction Mastermix
5 μL	Sample's nucleic acid
20 μL	Total volume of reaction

E. Close and briefly centrifuge the PCR tubes.

F. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm for a longer time.

Note: It is recommended to centrifuge PCR tubes before PCR to eliminate air bubbles and collect all residual liquids at the bottom of tubes.



Note: Use a new sterile pipette tip for each sample.

Note: For **Negative Control (NC)**, use 5 μL of "**RNase-free Water"** instead of sample's nucleic acid.

Note: For **Positive Control (PC)**, use 5 μL of "**SARS2 PC"** instead of sample's nucleic acid.

Note: Be careful not to cross-contaminate the Reaction Mastermix and samples with the Positive Control.

Note: Do not label the reaction tube on its cap. Fluorescence is detected from the top of each reaction tube.

REAL-TIME PCR INSTRUMENT SET UP AND RESULT ANALYSIS

1. CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6)

1.1. Real-time PCR Instrument Setup

Note: CFX96™ Real-time PCR Detection System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start run.

A. Protocol Setup

1) In the main menu, select **"File"** → **"New"** → **"Protocol"** to open **"Protocol Editor"**.

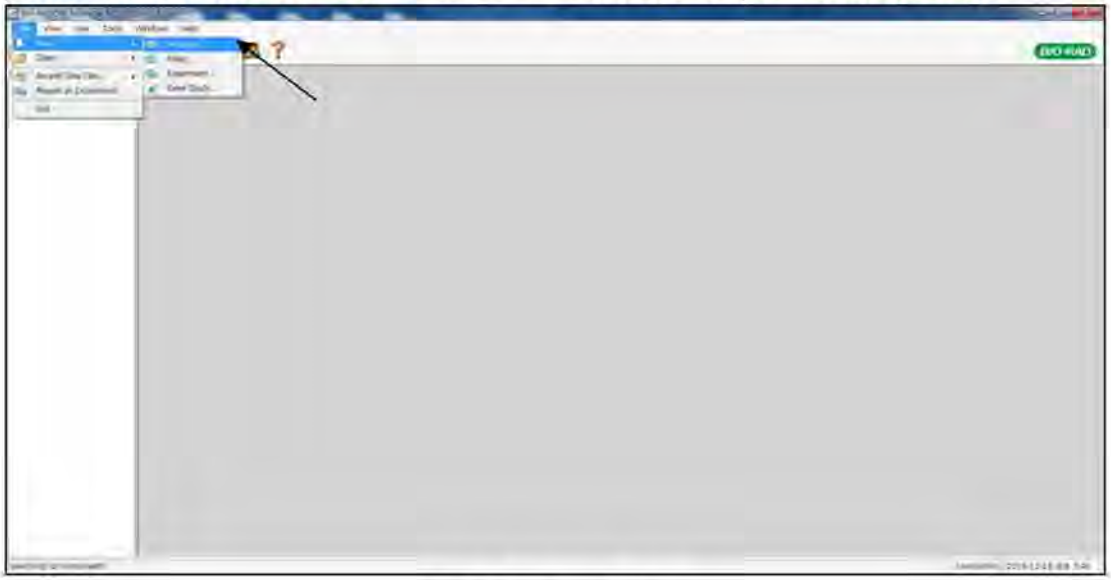


Fig. 1. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	95°C	10 sec
4*		60°C	15 sec
5*		72°C	10 sec

Note*: Plate Read at Step 4 and 5. Fluorescence is detected at 60°C and 72°C.

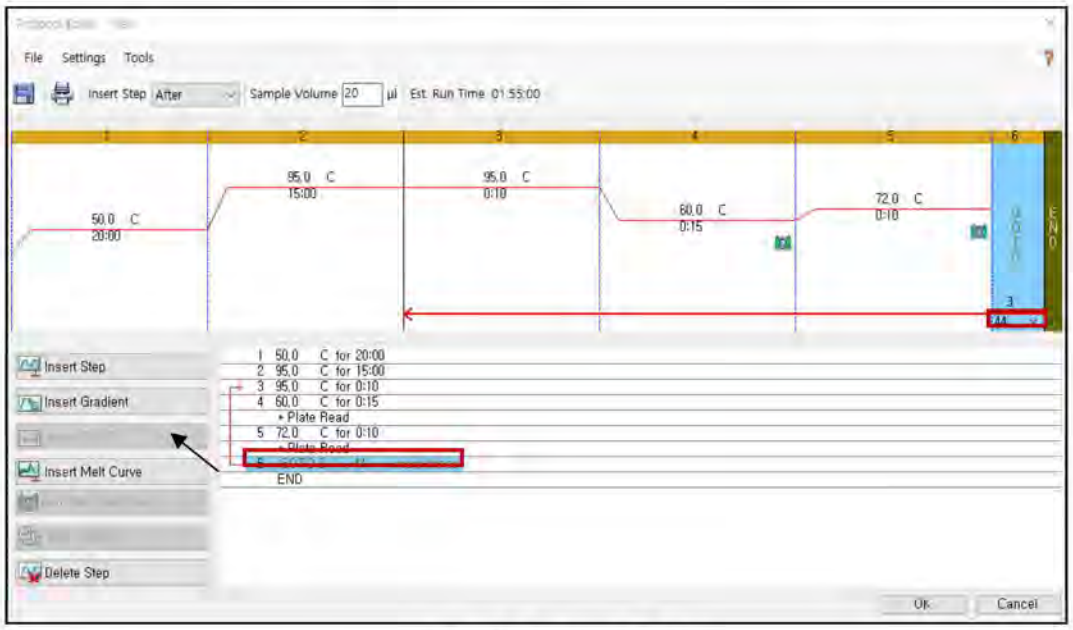


Fig. 2. **Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 3, 44 more times**" at Step 6.

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click **"OK"** and save the protocol to open the **"Experiment Setup"** window.

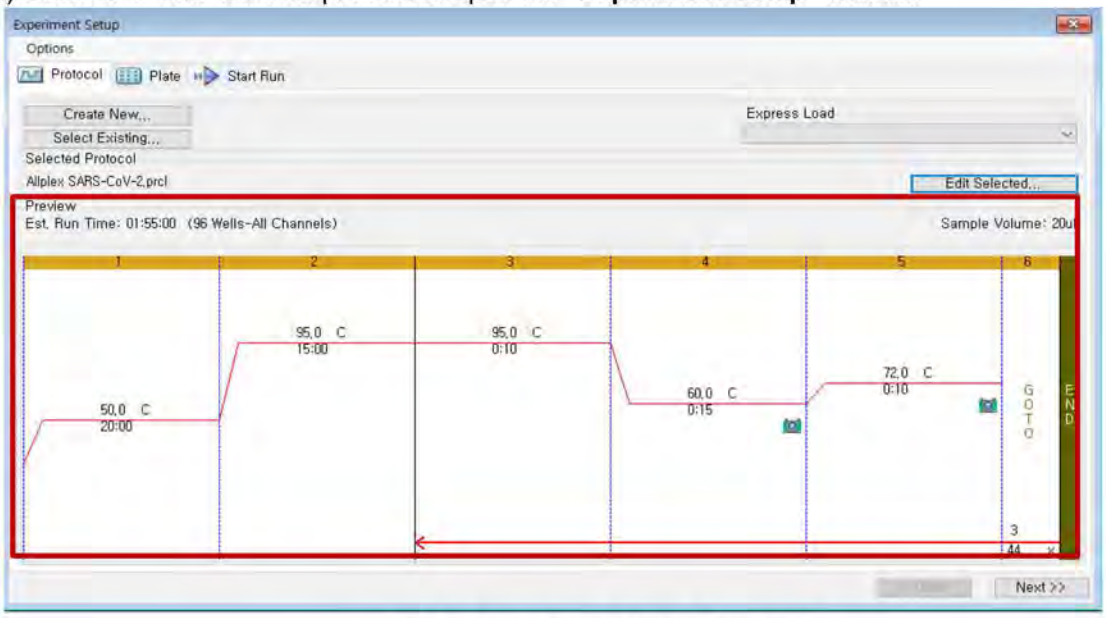


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

1) From **"Plate"** tab in **"Experiment Setup"**, click **"Create New"** to open **"Plate Editor"** window.

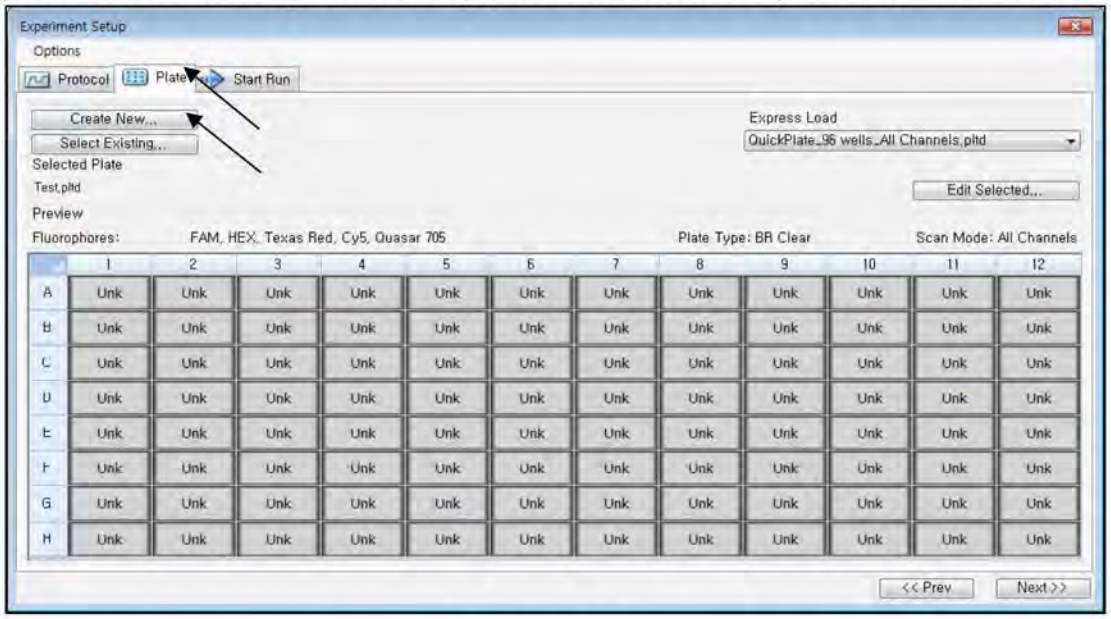


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670***) that will be used and click **"OK"**.

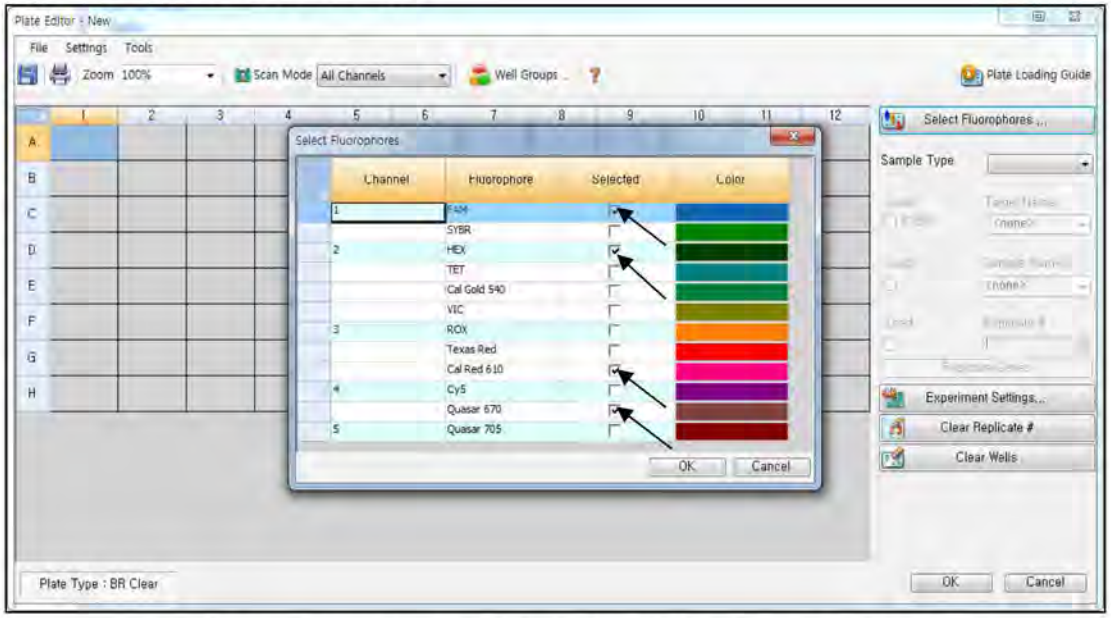


Fig. 5. **Select Fluorophores** (***FAM***, ***HEX***, ***Cal Red 610***, *and* ***Quasar 670***)

3) Select the wells where the PCR tube will be placed and select their sample types from the **"Sample Type"** drop-down menu.

- ***Unknown***: *Clinical samples*
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM, HEX, Cal Red 610, and Quasar 670***) to specify the fluorophores to be detected in the selected wells.

5) Type **"Sample Name"** and press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (*96 wells*)** and **"Plate Type" (*BR White*).**

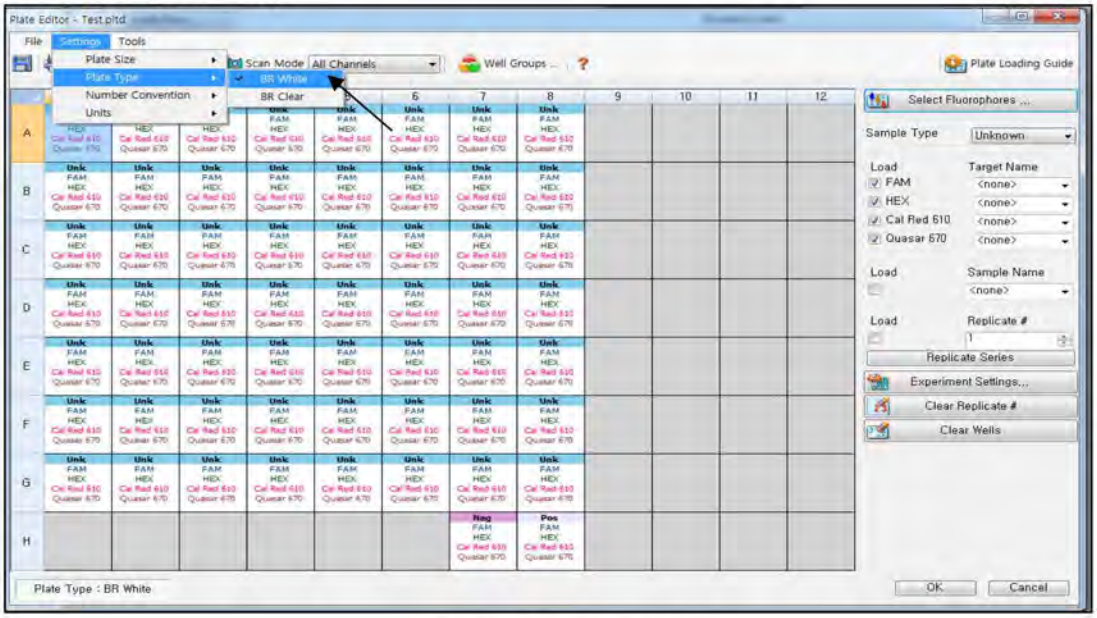


Fig. 6. **Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Experiment Setup"** window.

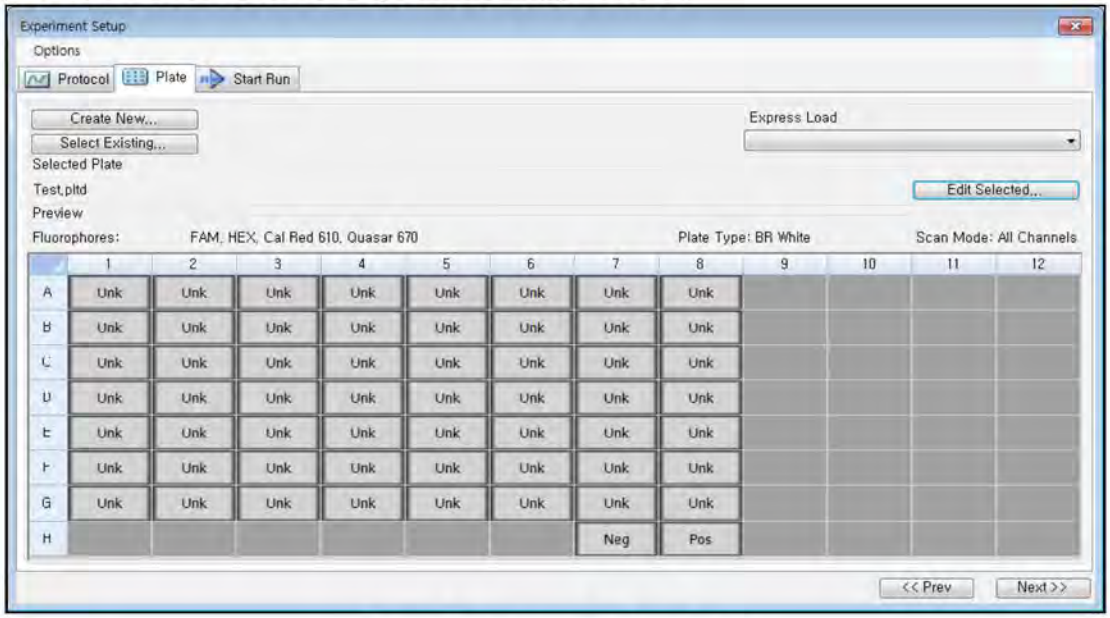


Fig. 7. **Experiment Setup: Plate**

9) Click **"Next"** to Start Run.

C. Start Run

1) From **"Start Run"** tab in **"Experiment Setup"**, click **"Close Lid"** to close the instrument lid.

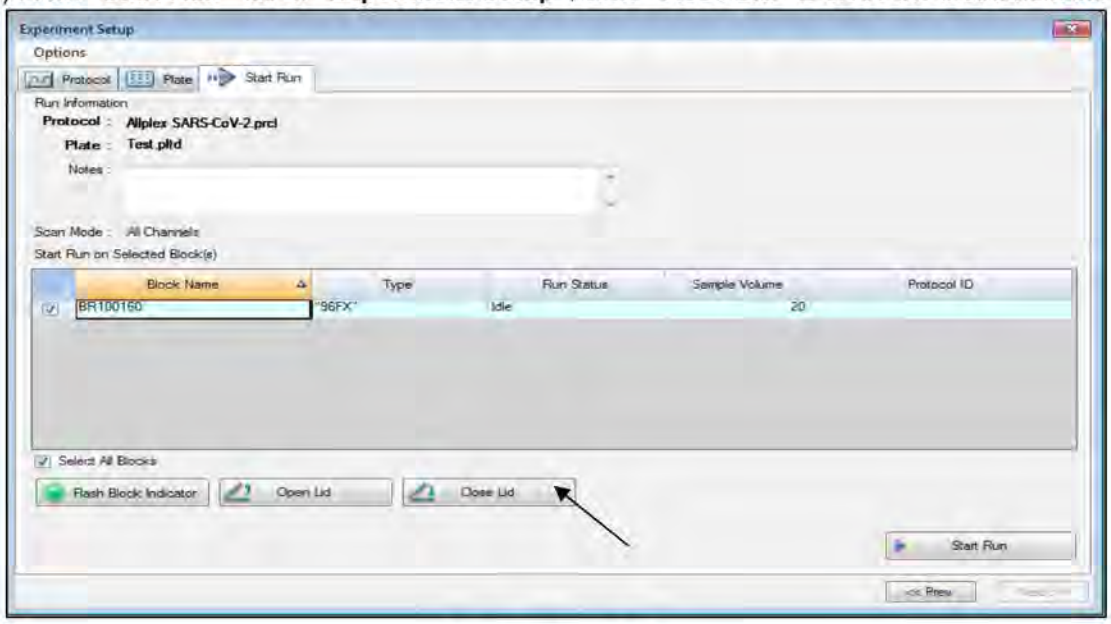


Fig. 8. **"Close Lid"**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

1.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantitation"** tab to see the amplification curve results.

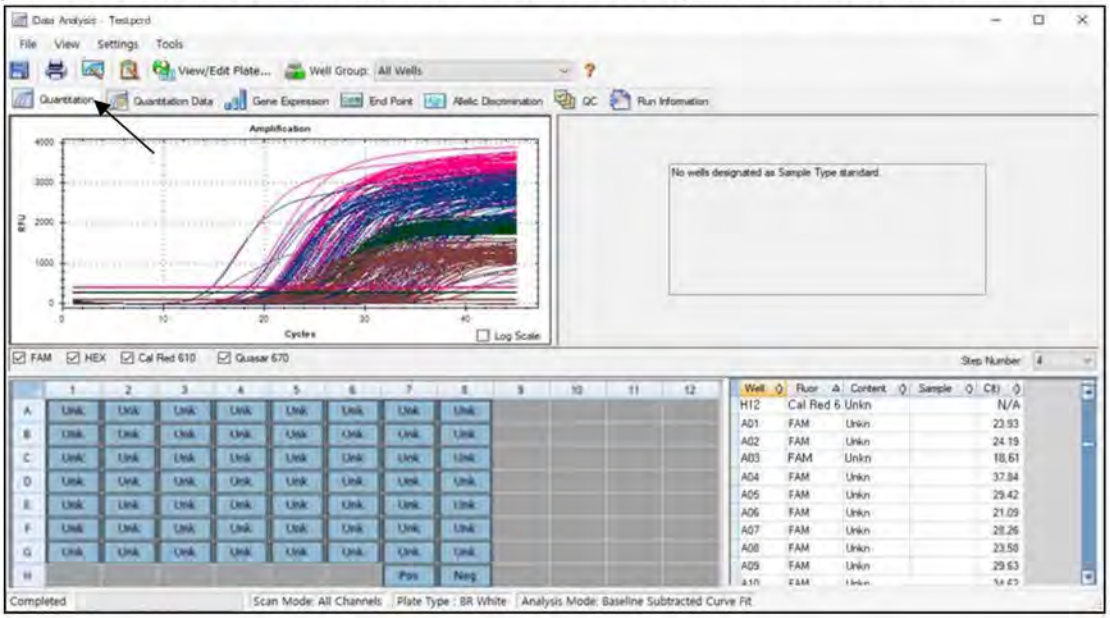


Fig. 9. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Analysis Mode of Settings menu.

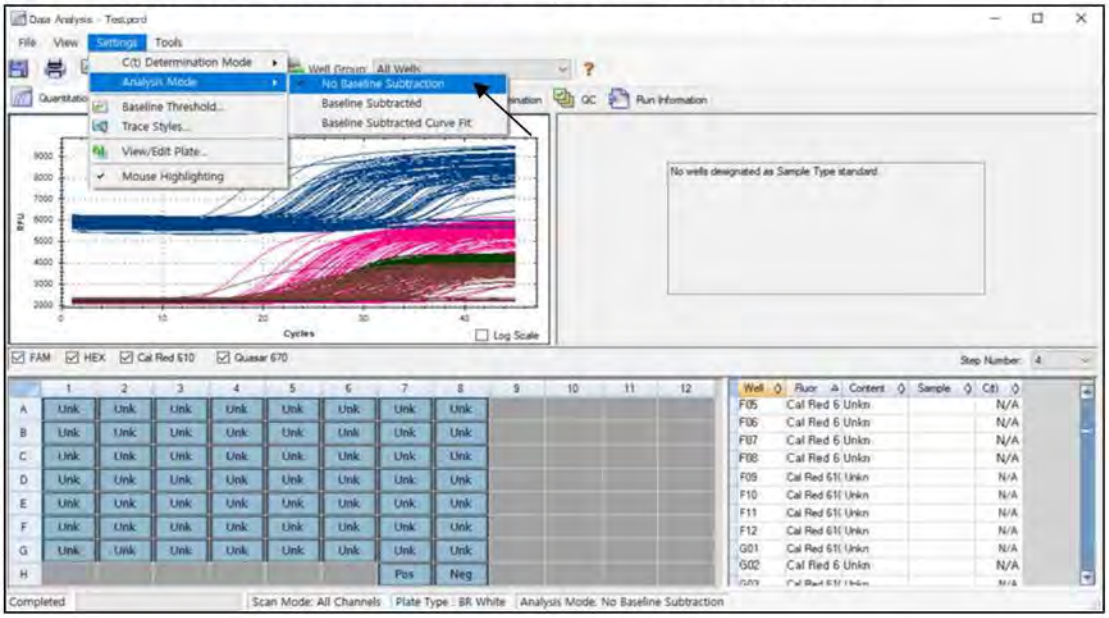


Fig. 10. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Tools menu.

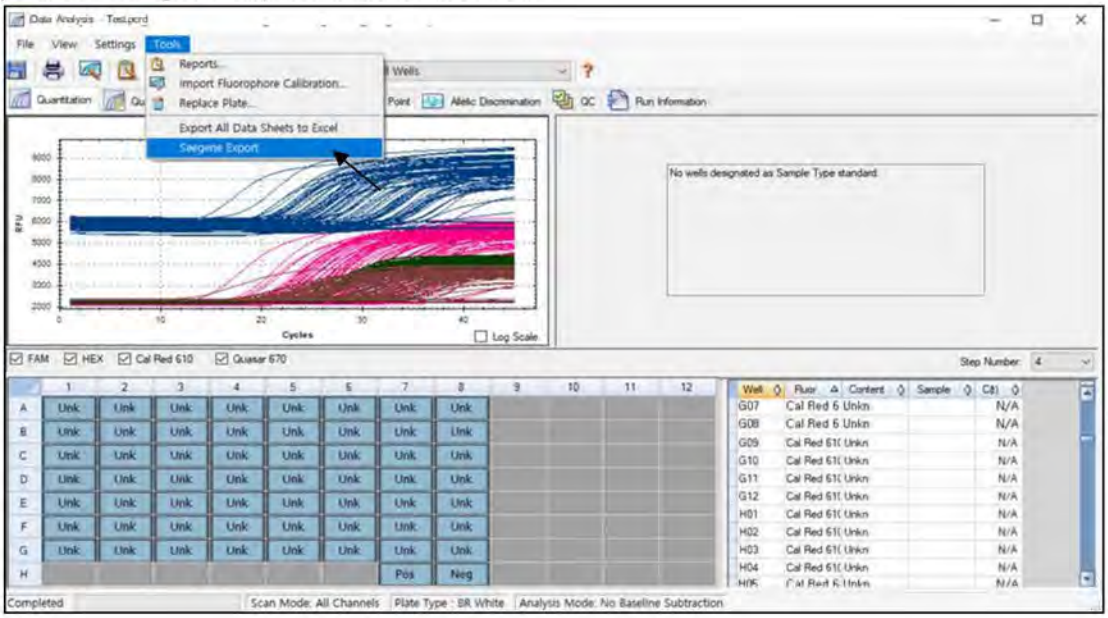


Fig. 11. **Seegene Export**

4) Choose a location to save data and click **"OK"**.

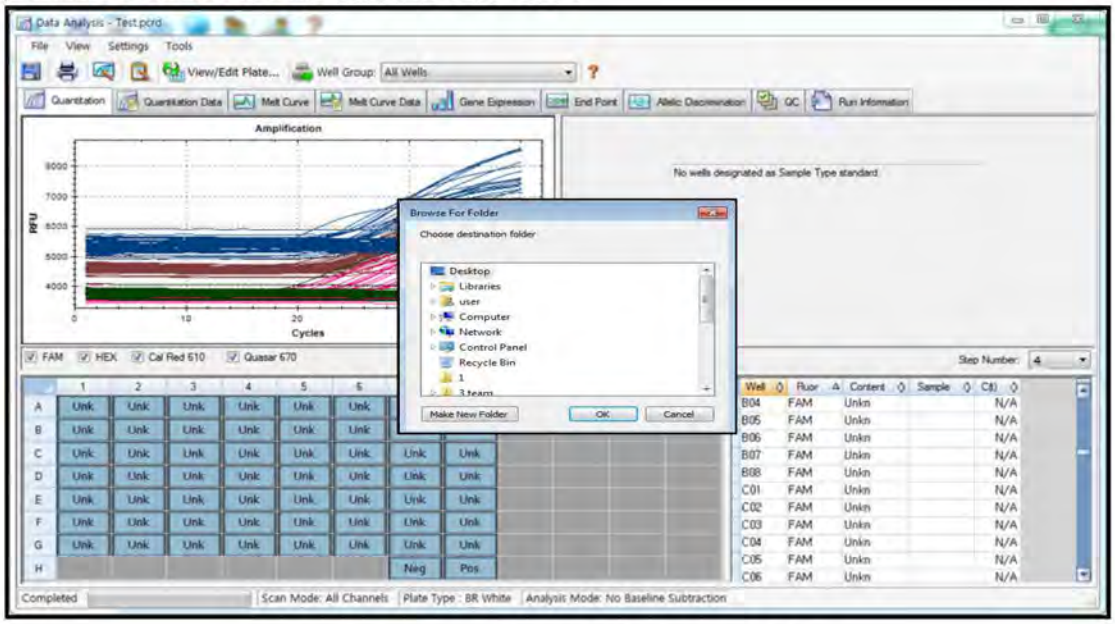


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program, and click **"Option"** to select ***CFX96 or CFX96 Dx*** in the **"Instrument"**.

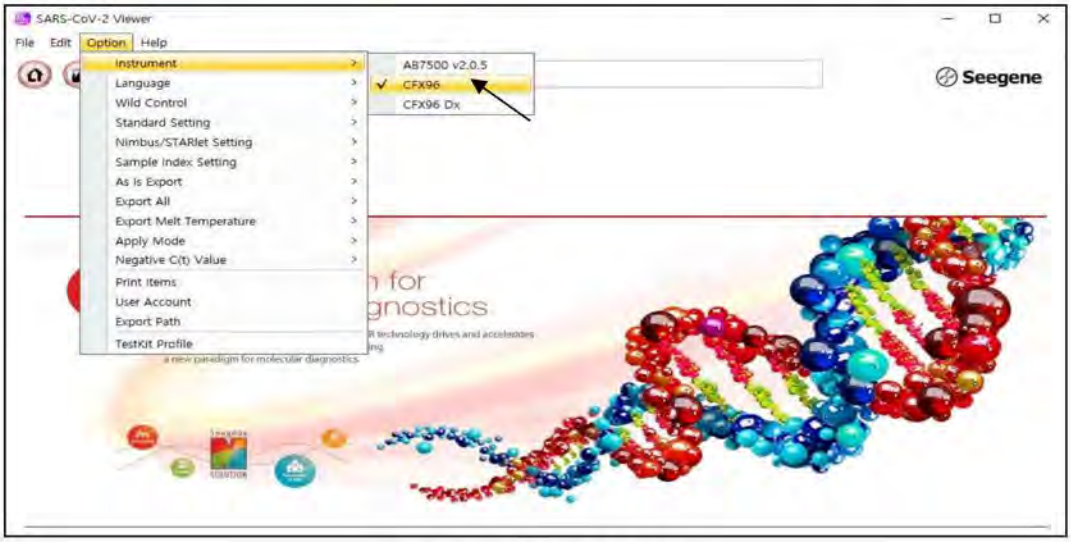


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep4", open the results file, and select the test kit from the **"PRODUCT"** menu.

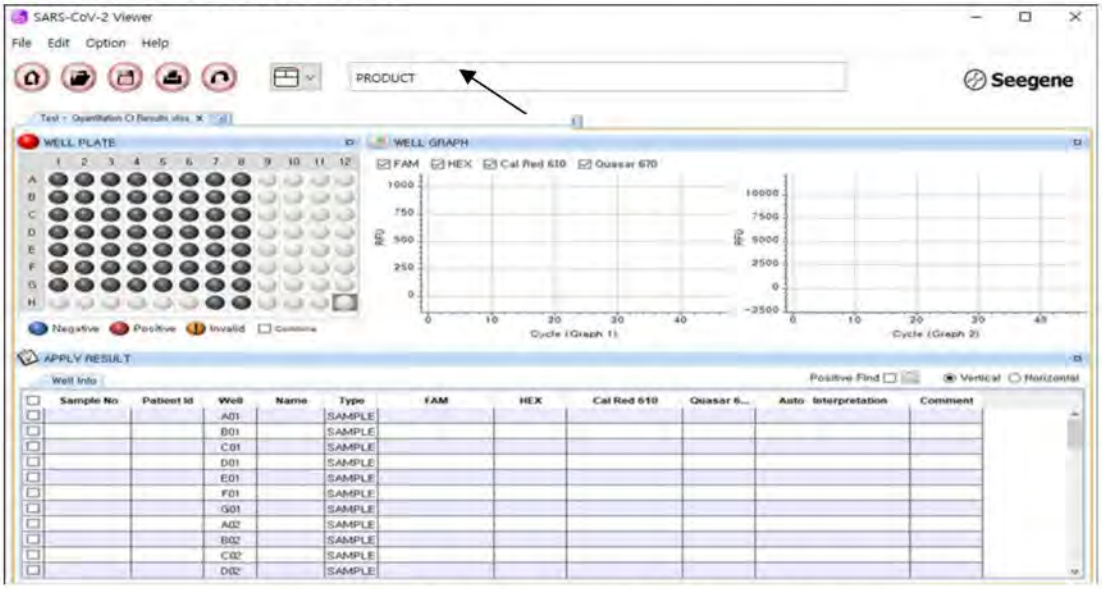


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

Note: In case of extraction-free method is applied, select "Allplex™ SARS-CoV-2 Assay (extraction-free)" from the PRODUCT menu.

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To check the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

1) Standard extraction

Control	Seegene Viewer Result				
	FAM (C_t)	Cal Red 610 (C_t)	Quasar670 (C_t)	HEX (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	Negative Control(-)

2) Extraction-free

Control	Seegene Viewer Result				
	FAM (C_t)	Cal Red 610 (C_t)	Quasar670 (C_t)	HEX (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	≤ 40	Negative Control(-)

b. Invalid Assay Run

In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

2. CFX96™ Dx System (CFX Manager™ Dx v3.1)

2.1 Real-time PCR Instrument Setup

Note: CFX96™ Dx System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start Run.

A. Protocol Setup

1) In the main menu, select **"File"** → **"New"** → **"Protocol"** to open **"Protocol Editor"**.

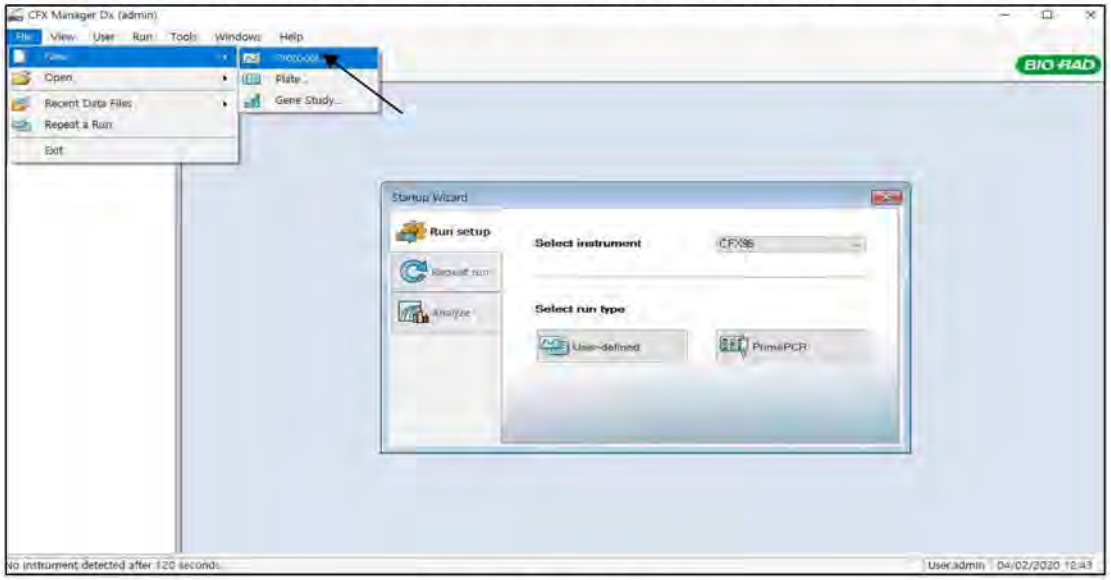


Fig. 16. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	95°C	10 sec
4*		60°C	15 sec
5*		72°C	10 sec

Note*: Plate Read at Step 4 and 5. Fluorescence is detected at 60°C and 72°C.

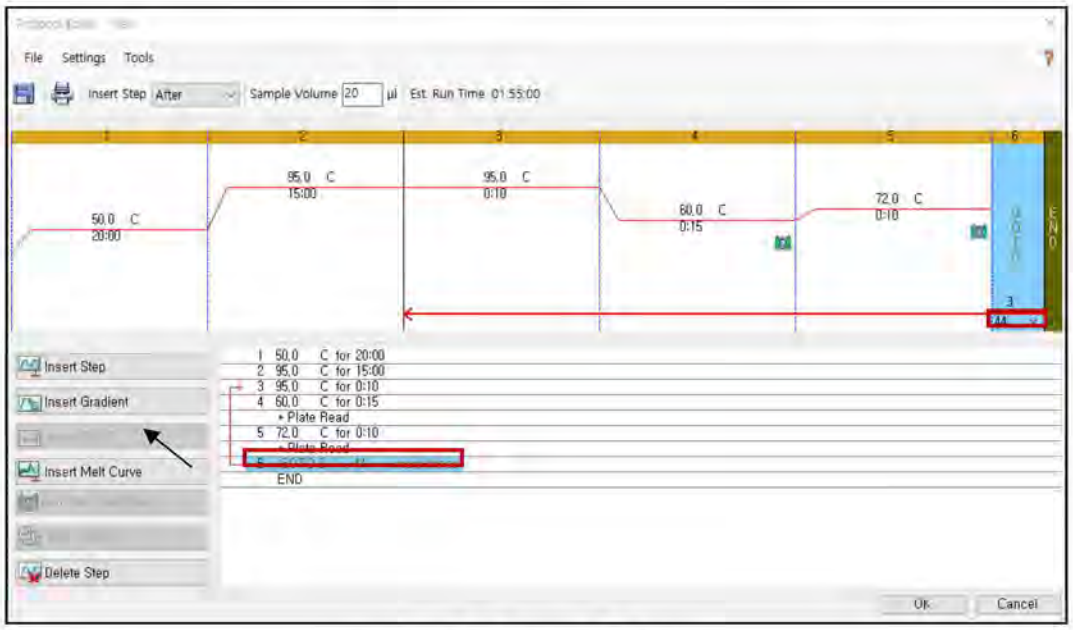


Fig. 17. **Protocol Editor**

Note: Click the "**Insert GOTO**" and type in "**GOTO 3, 44 more times**" at Step 6.

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click **"OK"** and save the protocol to open the **"Run Setup"** window.

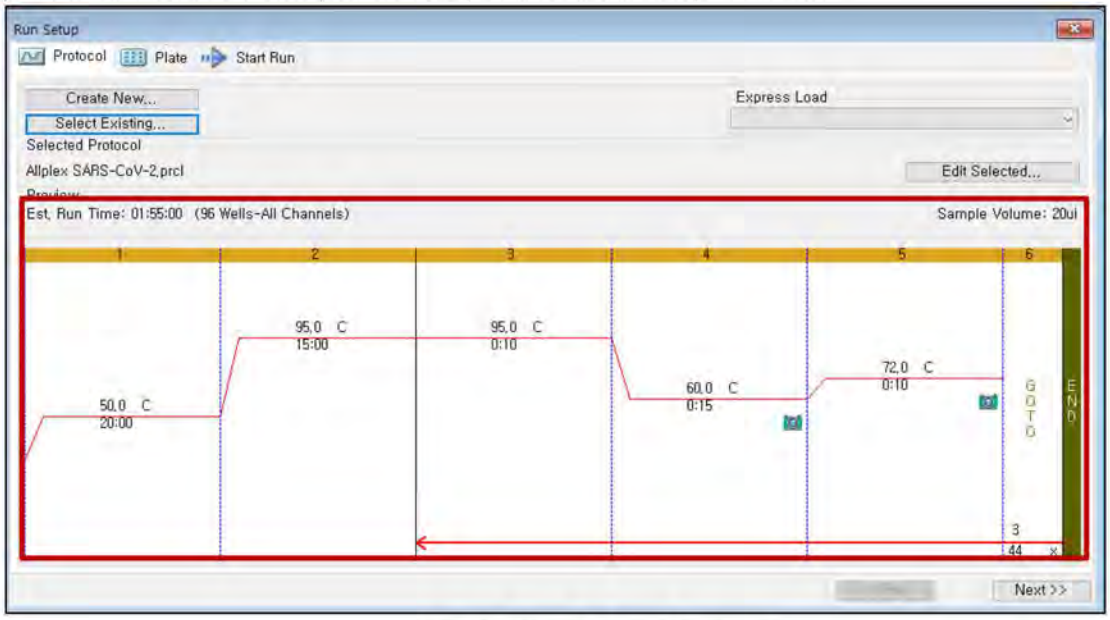


Fig. 18. **Run Setup: Protocol**

B. Plate Setup

1) From **"Plate"** tab in **"Run Setup"**, click **"Create New"** to open **"Plate Editor"** window.

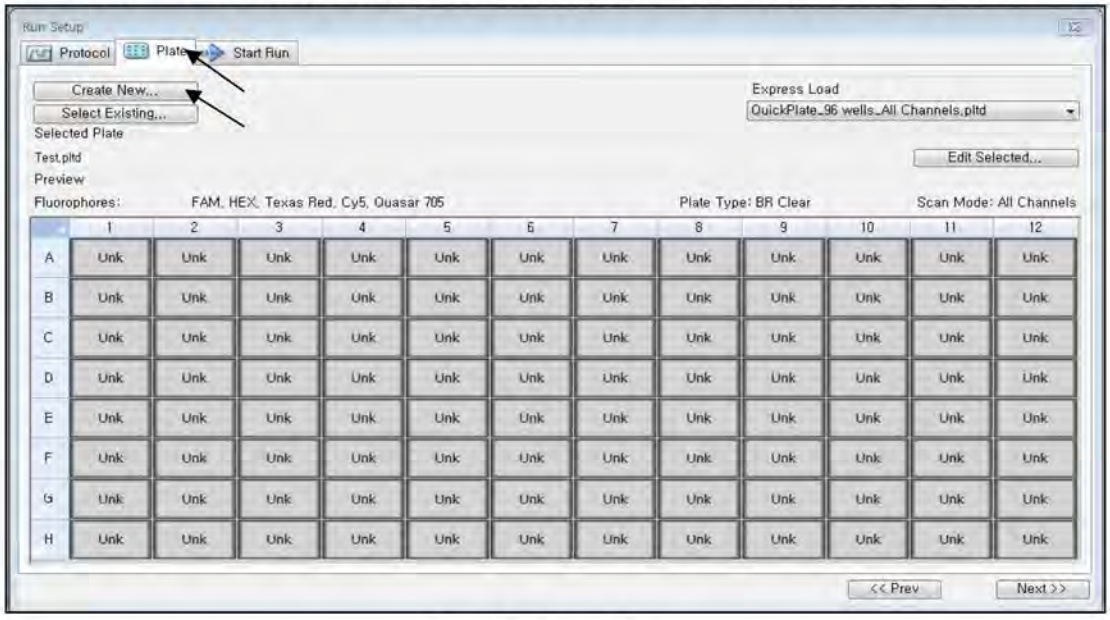


Fig. 19. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM***, ***HEX***, ***Cal Red 610***, ***Quasar 670***) that will be used and click **"OK"**.

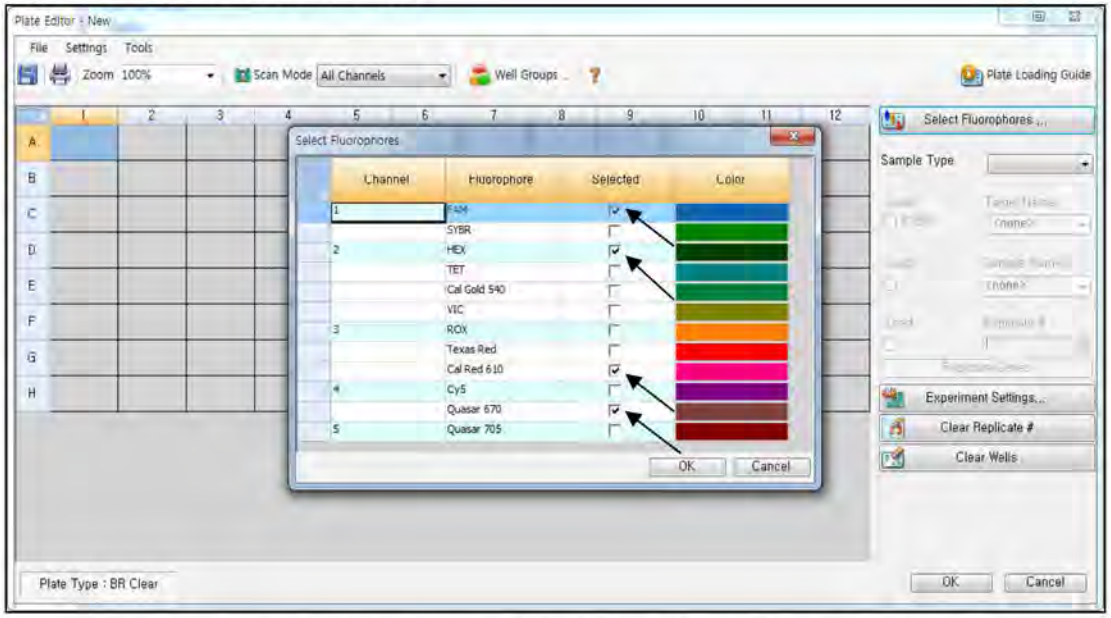


Fig. 20. **"Select Fluorophores"** (***FAM***, ***HEX***, ***Cal Red 610***, *and* ***Quasar 670***)

3) Select the wells where the PCR tube will be placed and select their sample types from the **"Sample Type"** drop-down menu.

- ***Unknown***: *Clinical samples*
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM, HEX, Cal Red 610, and Quasar 670***) to specify the fluorophores to be detected in the selected wells.

5) Type **"Sample Name"** and press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (*96 wells*)** and **"Plate Type" (*BR White*).**

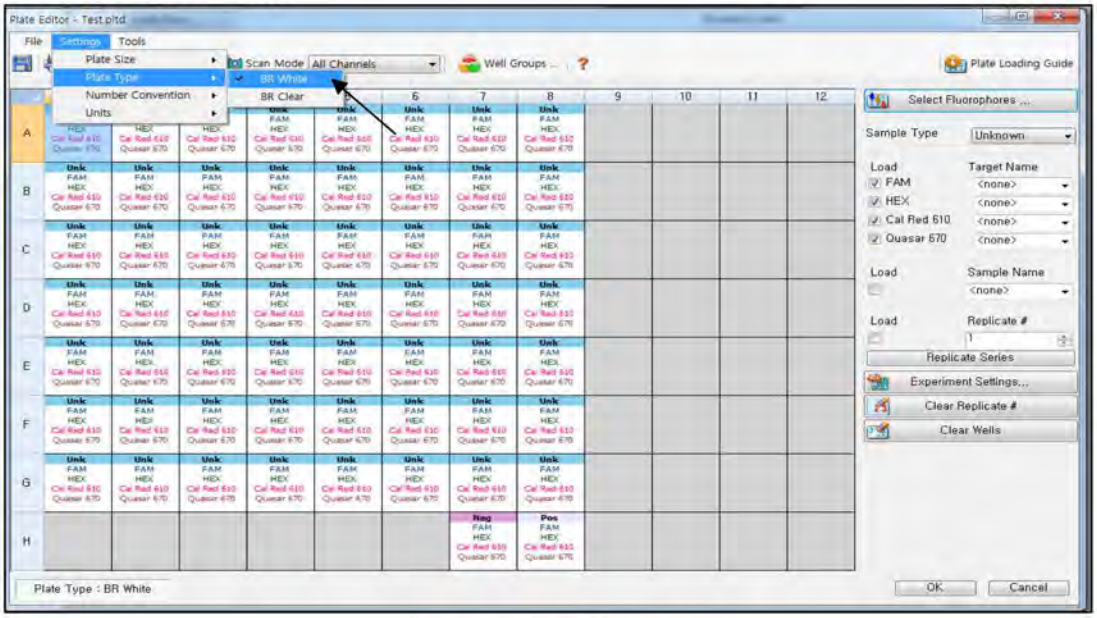


Fig. 21**. Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Run Setup"** window.

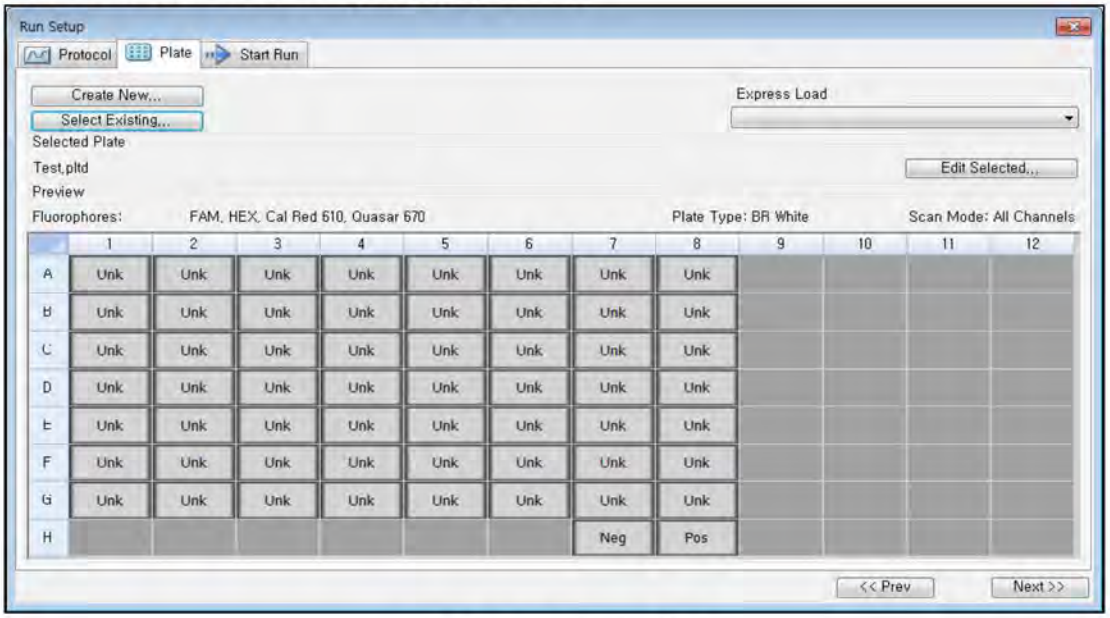


Fig. 22**. Run Setup: Plate**

9) Click **"Next"** to Start Run.

C. Start Run

1) From **"Start Run"** tab in **"Run Setup"**, click **"Close Lid"** to close the instrument lid.

Fig. 23. **Close Lid**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

2.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep4" and "QuantStep5" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantification"** tab to see the amplification curve results.

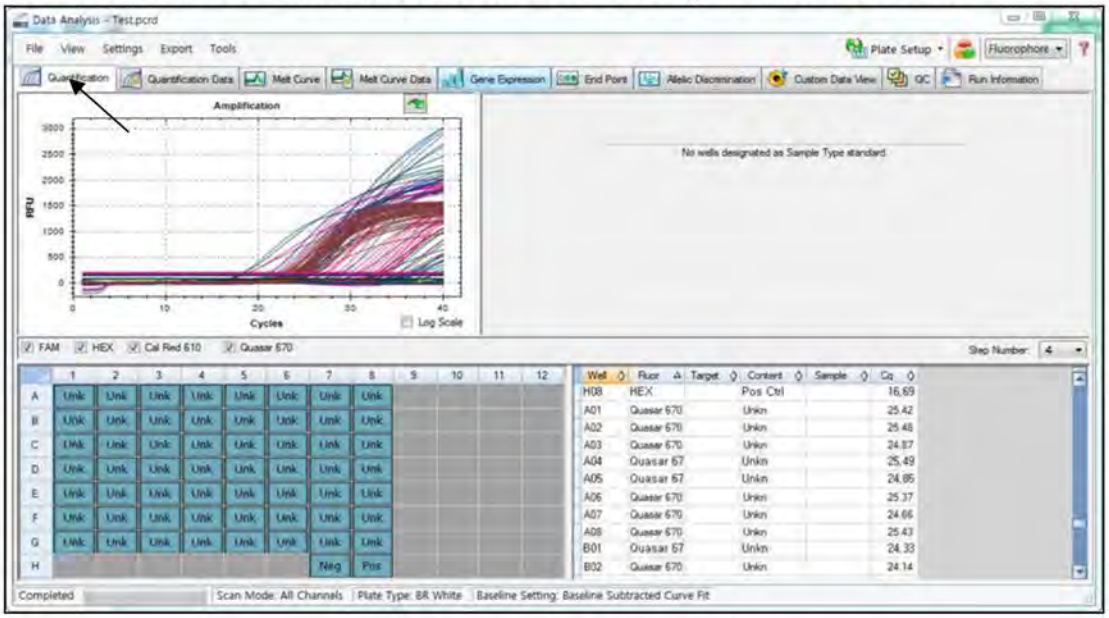


Fig. 24. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Baseline Setting of Settings menu.

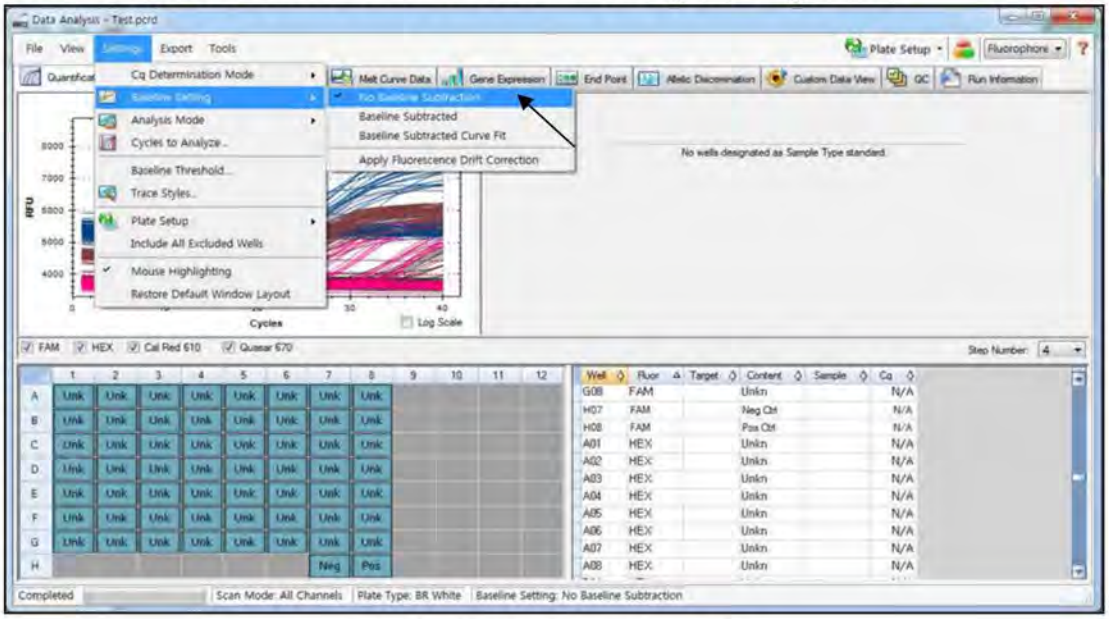


Fig. 25. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Export menu.

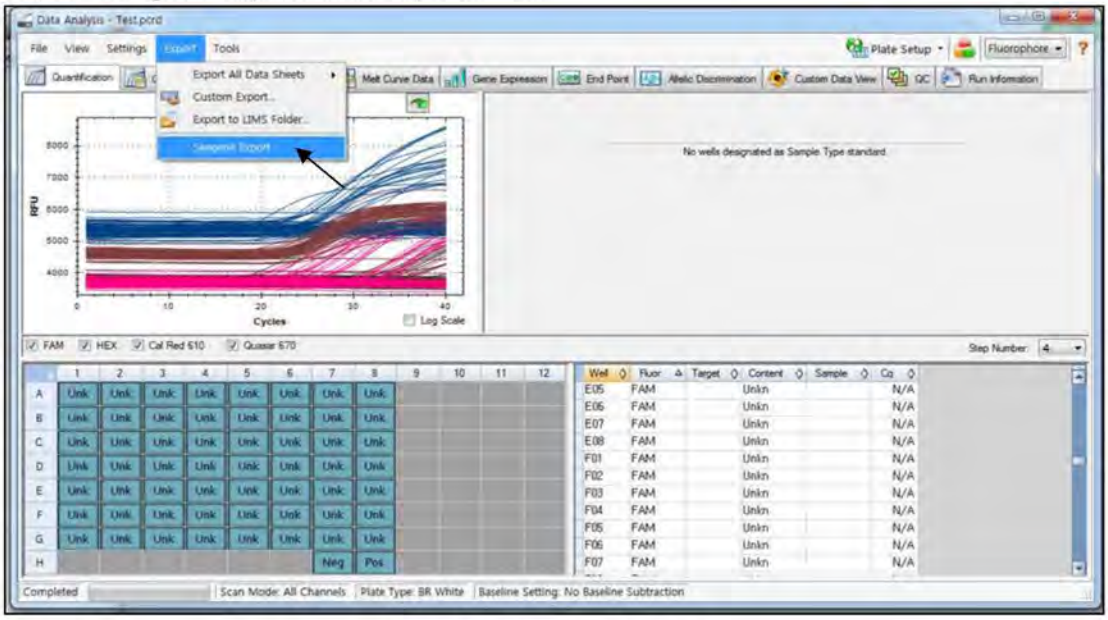


Fig. 26. **"Seegene Export"**

4) Choose a location to save data and click **"OK"**.

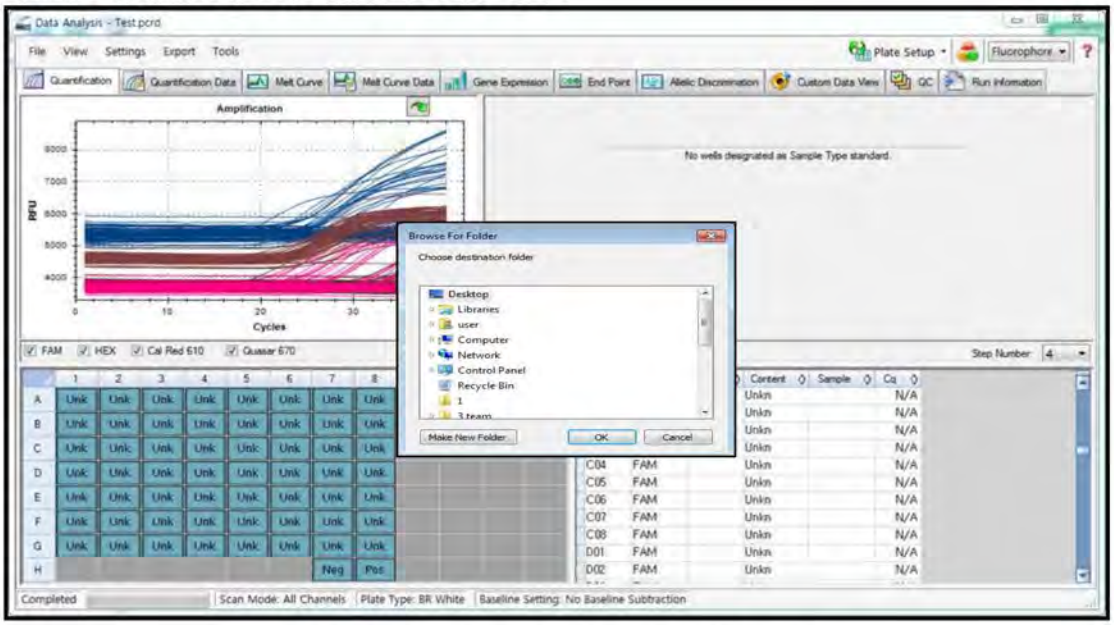


Fig. 27. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program, and click **"Option"** to select ***CFX96 or CFX96 Dx*** in the **"Instrument"**.

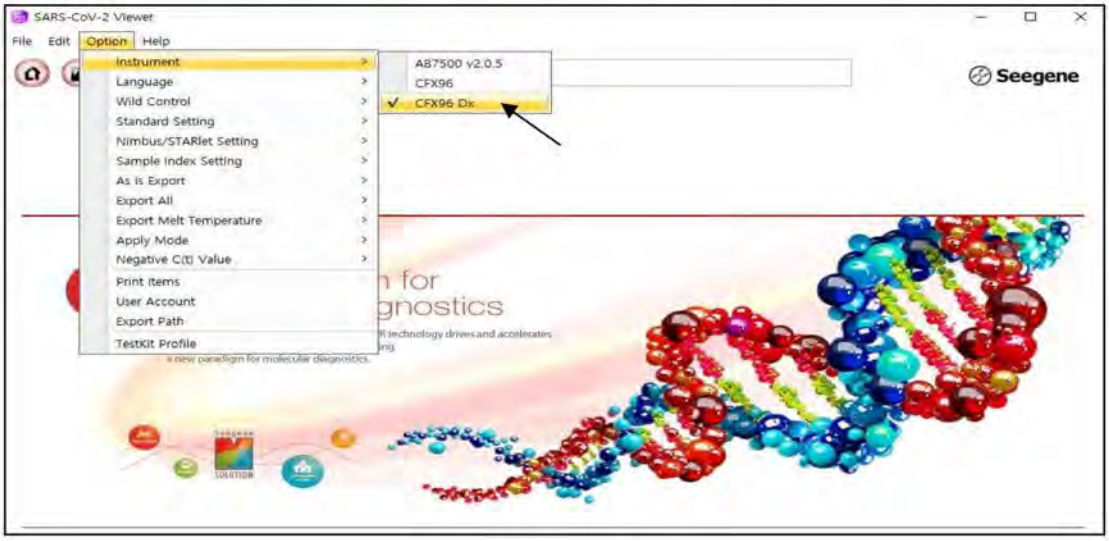


Fig. 28. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep4", open the results file, and select the test kit from the **"PRODUCT"** menu.

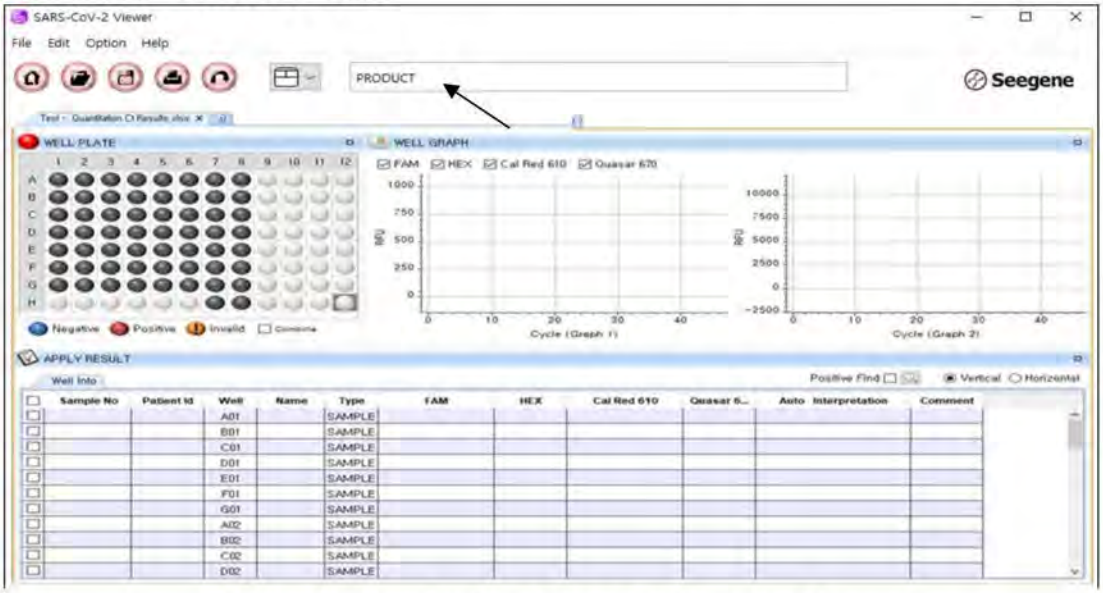


Fig. 29. **Settings for Data Analysis in Seegene Viewer**

Note: In case of extraction-free method is applied, select "Allplex™ SARS-CoV-2 Assay (extraction-free)" from the PRODUCT menu.

3) Check the result for each well.

Fig. 30. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To check the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

1) Standard extraction

Control	Seegene Viewer Result				
	FAM (C_t)	Cal Red 610 (C_t)	Quasar670 (C_t)	HEX (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	Negative Control(-)

2) Extraction-free

Control	Seegene Viewer Result				
	FAM (C_t)	Cal Red 610 (C_t)	Quasar670 (C_t)	HEX (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	≤ 40	Negative Control(-)

b. Invalid Assay Run

In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

3. Applied Biosystems™ 7500 (SDS software v2.0.5)

3.1. Real-time PCR Instrument set up

Note: The instrument must be calibrated before use.

Note: Applied Biosystems™ 7500(Thermo Fisher Scientific) experiment setup can be divided into two steps: Setup and Run

A. Setup

1) In the main menu, select **"Set Up"** → **"Advanced Setup"**

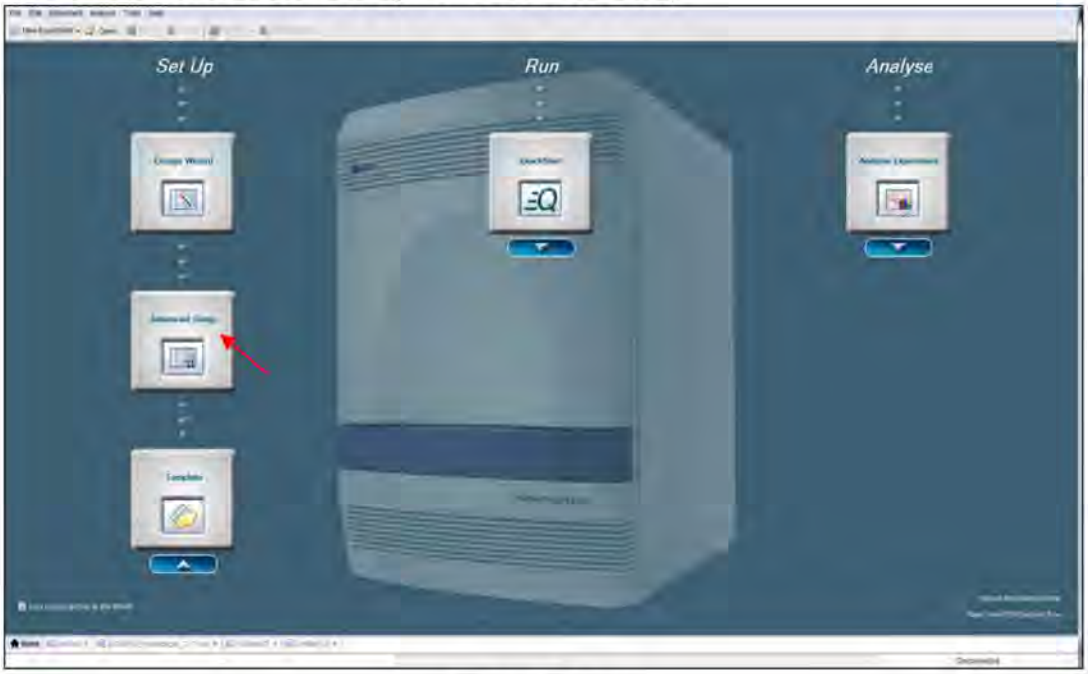


Fig 31. **Set up**

2) In the **"Experiment properties"** tab, enter **"Experiment Name"** and select Instrument, **"Experiment type"**, **"Reagents"**, and **"Ramp speed"** as follows.

Instrument	7500 (96 Wells)
Experiment type	Quantitation – Standard Curve
Reagents	Taqman® Reagents
Ramp speed	Standard

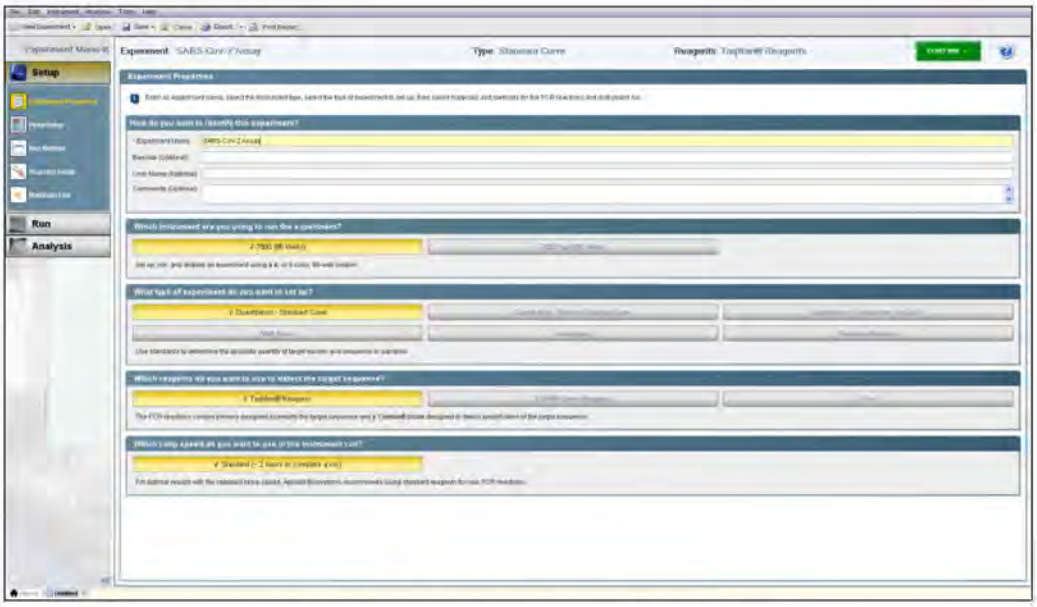


Fig. 32. **Experiment properties tab**

3) Click on **"Plate setup"** tab. In the **"Define Targets and Samples"** tab, enter **"Target Name"** and select **"Reporter"** and **"Quencher"** as follows.

Target Name	Reporter	Quencher
E gene	FAM	None
IC	VIC	None
RdRP/S gene	ROX	None
N gene	CY5	None

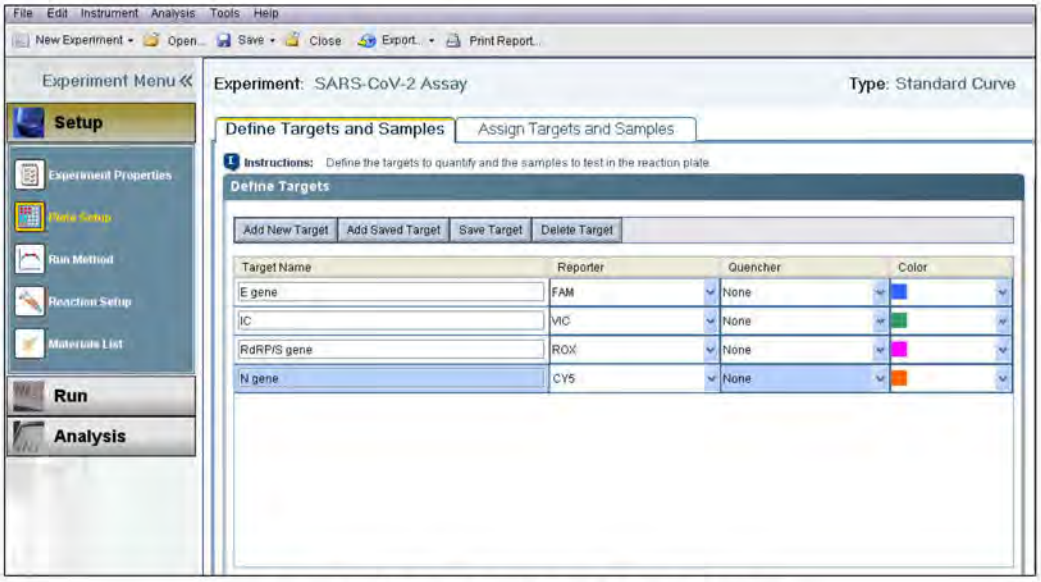


Fig. 33. **Define Targets and Samples tab**

4) Click on "**Assign Targets and Samples"** tab, select wells where the PCR tube will be placed and assign targets. Select None for Passive reference.

NOTE: If a well without sample or mastermix is selected, signal noise may be observed. Ensure that only wells containing samples or mastermix are selected.

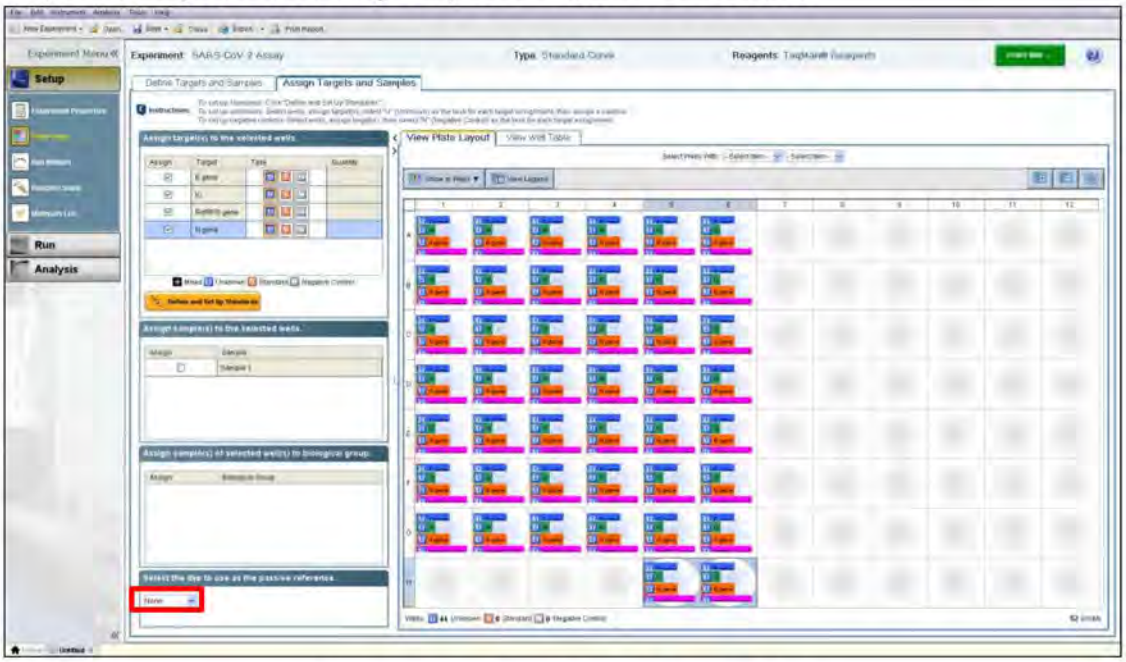


Fig. 34. **Assign Targets and Samples tab**

5) Click on "**Run Method"**. In the "**Graphical View"** or "**Tabular View tab"**, enter 20 µL as the "**Reaction Volume per Well"** field. Define the thermal profile as table below.

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	45	95°C	10 sec
4*		60°C	30 sec
5		72°C	10 sec

Note*: Plate Read at Step 4. Fluorescence is detected at 60°C.

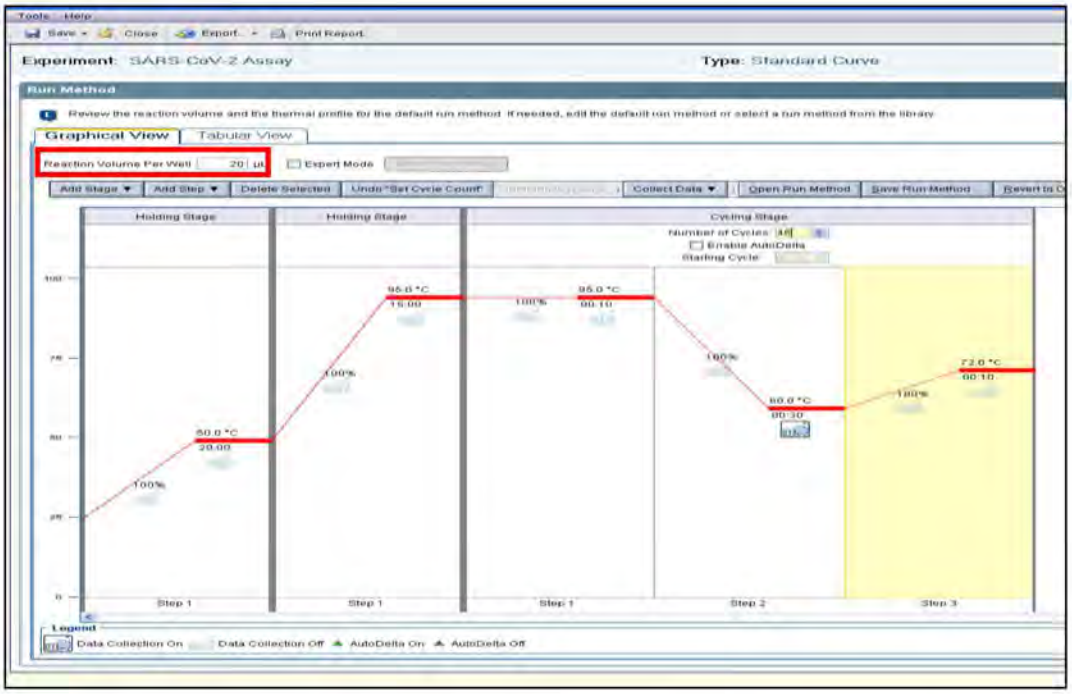


Fig. 35. **Graphical View tab**

6) Click on **"File"** → **"Save as Template"** to save the new **"template"** file in **".edt"** format. Enter the file name, select a location for the template, then click **"Save"**. The saved **"template"** can be used for future testing.

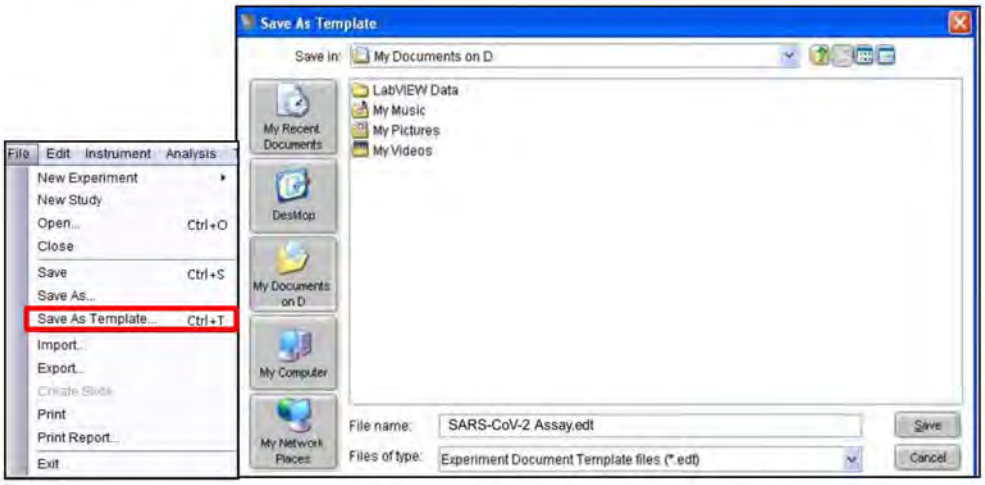


Fig. 36. **Save as Template (.edt)**

B. Run

1) Turn on the laptop and Applied Biosystems™ 7500 real-time PCR system. Ensure that the laptop is connected to the instrument.
2) Push the tray door to open the instrument. Load the PCR plate onto the plate holder of the instrument.
3) Push the tray door to close the instrument.
4) Click on **"File"** → **"Save as Template"** to save the new template file in **".eds"** format. Enter the file name, select a location for the template, then click **"Save"**.

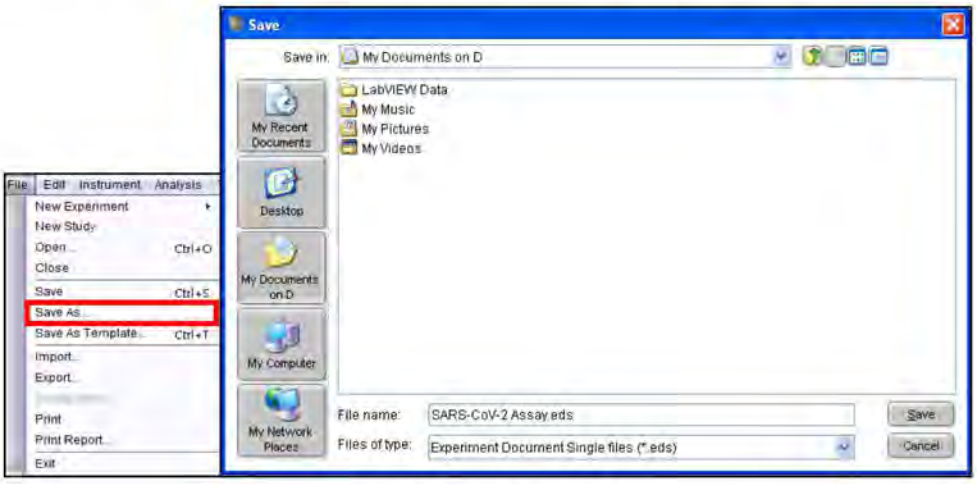


Fig. 37. **Save as (.eds)**

5) Click **START RUN.**

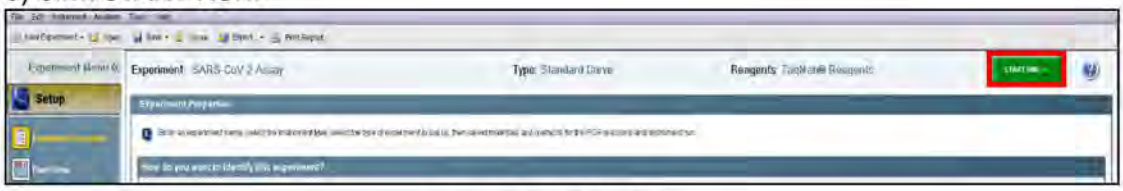


Fig. 38. **START RUN**

3.2. Data export and analysis

A. Pre-settings for Data export and analysis

1) Create a folder to save data for all of amplification curve detection steps from the result file.
2) Enter folder name as necessary.

3) Click on **"File" → "Export"**

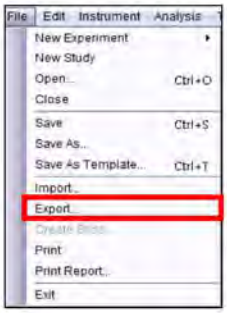


Fig. 39. **File export**

4) Click on the "**Export Properties**" tab (default) and select "**Sample Setup**", "**Raw data**", "**Amplification Data**", "**Results**", and "**Multicomponent Data**" under "**1. Select data to export**".

Fig. 40. **1. Select data to export**

5) Select **"One File"** under "**2. Select one or separate files:**".

Fig. 41. **2. Select one or separate files**

6) Enter **"Export File Name"**, then select **"Export File Location"**. Select **".xls"** in the **"File Type"** drop-down list.

Fig. 42. **3. Enter export file properties**

7) Click **"Start Export".**

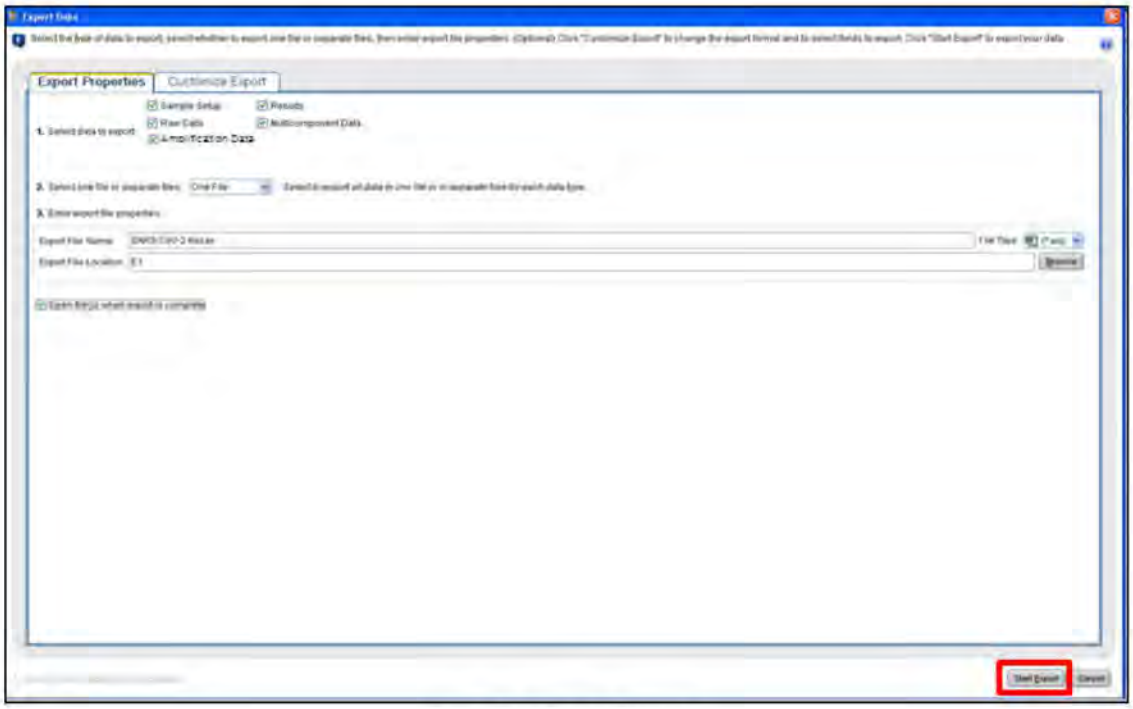


Fig. 43. **Start Export**

B. Set up for Data analysis in Seegene Viewer

1) Open the Seegene Viewer software installed on the laptop connected to the Applied Biosystems™ 7500. Click on **"Option"** to select AB7500 v2.0.5 from the **"Instrument menu"**.

Fig. 44. **Seegene Viewer**

2) Click on the **"Open"** icon and locate the Applied Biosystems™ 7500 export data where the Applied Biosystems™ 7500 data was saved. After opening the results file, select 'Allplex™ SARS-CoV-2 Assay' from the PRODUCT menu.

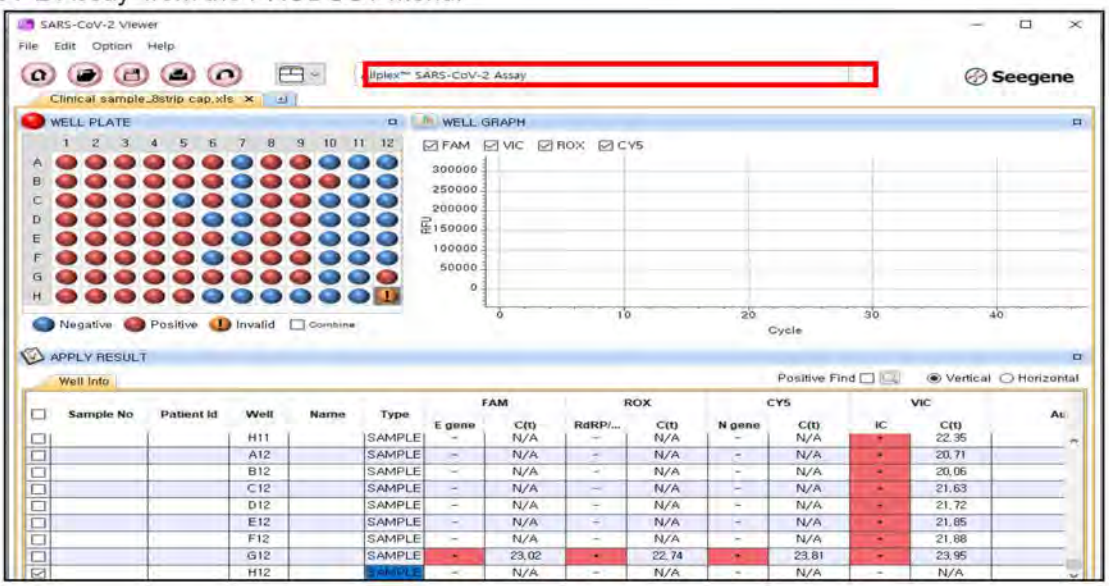


Fig. 45. **Settings for Data Analysis in Seegene Viewer**

Note: In case of extraction-free method is applied, select "Allplex™ SARS-CoV-2 Assay (extraction-free)" from the PRODUCT menu.

3) Assign Positive and Negative control accordingly by selecting PC, NC under the Type drop-down menu.

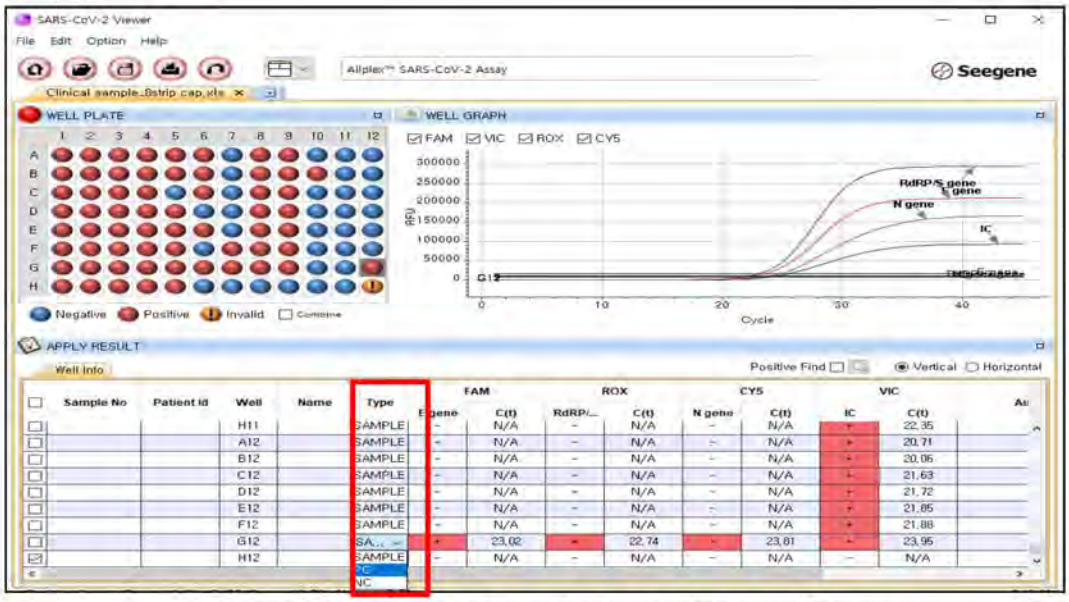


Fig. 46. **Settings for Sample Type in Seegene Viewer**

4) View test results. The auto-interpreted results for each sample can be viewed by clicking on each well.

5) Validity Criteria of Control Results

a. Valid Assay Run

To check the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

1) Standard extraction

Control	Seegene Viewer Result				
	FAM (C_t)	ROX (C_t)	CY5 (C_t)	VIC (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	Negative Control(-)

2) Extraction-free

Control	Seegene Viewer Result				
	FAM (C_t)	ROX (C_t)	CY5 (C_t)	VIC (C_t)	Auto Interpretation
	E gene	RdRP/S gene	N gene	IC	
Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	≤ 40	Negative Control(-)

b. Invalid Assay Run

In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

RESULTS

1. Analyte Information

CFX96	AB7500	Analytes
Fluorophores	Fluorophores	
FAM	FAM	E gene
HEX	VIC	IC
Cal Red 610	ROX	RdRP gene, S gene
Quasar 670	CY5	N gene

2. Interpretation of Results

Analytes	C_t value	Result
Targets	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)
IC	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)

Target Result			IC Result*	Auto-interpretation	Description
E gene	RdRP/S gene	N gene			
+	+	+	+/-	SARS-CoV-2	All Target Results were valid. Result for SARS-CoV-2 RNA is detected.
+	-	+	+/-		- All Target Results were valid. Result for SARS-CoV-2 RNA is detected. - Negative target results are suggestive of 1) A sample at concentrations near or below the limit of detection of the test, 2) A mutation in the corresponding target region, 3) Other factors.
-	+	+	+/-		
+	+	-	+/-		
-	+	-	+/-		
-	-	+	+/-		
+	-	-	+/-	**SARS-CoV-2 Presumptive positive**	- All Target Results were valid. Result for Sarbecovirus RNA is detected. Result for SARS-CoV-2 RNA is Presumptive Positive. - Negative target results are suggestive of 1) A sample at concentrations near or below the limit of detection of the test, 2) A mutation in the corresponding target region, 3) Other factors. - Repeat test with more nucleic acid (up to 10ul) - For samples with the same result on the repeated test, additional confirmatory testing may be conducted, if it is necessary to

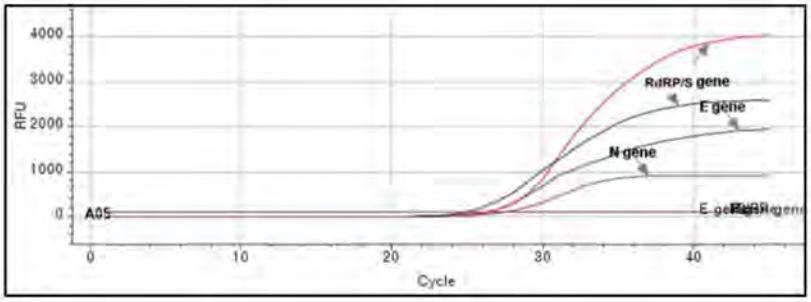
					differentiate between SARS-CoV-2 and other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
-	-	-	+	**Not detected (-)**	All target results were valid. Result for SARS-CoV-2 RNA is not detected.
-	-	-	-	**Invalid****	- Results suggest inadequate specimen collection or processes (*e.g.*, no exogenous IC added) or the presence of PCR inhibitors. - Repeat the test from the nucleic acid extraction using another aliquot of the original specimen. - If the same result is shown in the diluted nucleic acid, please collect samples again.

* High level of target nucleic acids may cause interference in Internal Control detection and readout. Invalid IC signal does not indicate that the positive results for targets are invalid.

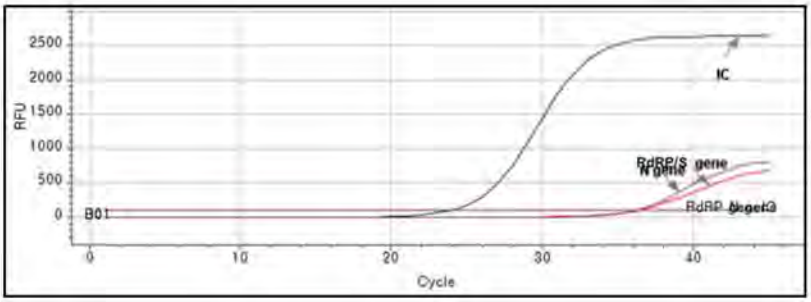
** See TROUBLESHOOTINGS section for the detailed instruction.

3. Application to Clinical Samples

Clinical Sample 1



Clinical Sample 2



CFX96	FAM		Cal Red 610		Quasar 670		HEX		Auto Interpretation
AB7500	FAM		ROX		CY5		VIC		
Sample	E gene	C(t)	RdRP/ S gene	C(t)	N gene	C(t)	IC	C(t)	
1	+	26.32	+	26.41	+	27.83	+	24.93	SARS-CoV-2
2	-	N/A	+	36.25	+	36.36	+	23.85	SARS-CoV-2

TROUBLESHOOTING

Allplex™ SARS-CoV-2 Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 8) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	If IC had been exogenously added to the specimen prior to extraction, the absence of IC signal may indicate loss of nucleic acids during the extraction. Make sure to use recommended extraction method. If due to inhibitors, re-extract the original specimen or the specimen may be diluted with saline buffer 1/3~1/10 fold and then add "RP-V IC 2" to the diluted specimen.
No Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not IC, then IC amplification may have been inhibited by high titer of target pathogen. If you want to observe IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.

Allplex™ SARS-CoV-2 Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative false positive or target signal(s) observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.
Putative False negative or no signal observed in Positive Control	Error in specimen collection	Please check the specimen collection method, and re-collect the specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
	Error in adding nucleic acid to correct PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect PCR mixture	Confirm that all components are added to the reaction mixture (Sensitivity is compromised with pre-composed premix). All reagents must be homogenized and spun down before use.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Centrifuge the PCR tube before run.
No signal from extraction-free method	Presence of PCR Inhibitor	Please check the specimen collection device. ENAT PM 2ML PERNASAL APPLICATOR, GeneTM Set (GTS2), GeneTM Set (GTS1), and SELTM Set (STS2) are not applicable to extraction-free method.

PERFORMANCE

1. Analytical Specificity

The high specificity of Allplex™ SARS-CoV-2 Assay is ensured by the oligos designed specifically for the targets of interest. Allplex™ SARS-CoV-2 Assay was tested for cross-reactivity to 75 different pathogens, and PCR amplification and detection were only identified for the specified targets.

NO.	Organism	Source	Isolate No.	Result†
1	SARS-CoV-2 isolate Australia/VIC01	TWIST BIOSCIENCE	102019	E, RdRP/S, N gene Detected
2	SARS-CoV-2 isolate Wuhan-Hu-1	TWIST BIOSCIENCE	102024	E, RdRP/S, N gene Detected
3	Human coronavirus HKU1	Korean isolate		Not Detected
4	Human coronavirus OC43	ATCC	VR-1558	Not Detected
5	Human coronavirus NL63	ZMC	0810228CF	Not Detected
6	Human coronavirus 229E	Korean isolate		Not Detected
7	SARS-coronavirus	ZMC	NATSARS-ST	E gene Detected
8	MERS-coronavirus	ZMC	NATMERS-ST	Not Detected
9	Influenza A virus (H1N1)	ATCC	VR-95 (H1N1)	Not Detected
10	Influenza A virus (H3N2)	ATCC	VR-547	Not Detected
11	Influenza B virus	ATCC	VR-523	Not Detected
12	Human Rhinovirus 1	KBPV	VR-81	Not Detected
13	Rhinovirus 21	KBPV	VR-40	Not Detected
14	Human rhinovirus type 90	ATCC	VR-1291	Not Detected
15	Human rhinovirus type 16	ATCC	VR-283	Not Detected
16	Human rhinovirus type 42	ATCC	VR-338	Not Detected
17	Human rhinovirus type 8	ATCC	VR-488	Not Detected
18	Human rhinovirus type 14	ATCC	VR-284	Not Detected
19	Human enterovirus type 68	ATCC	VR-1826	Not Detected
20	Human enterovirus type 70	ATCC	VR-836	Not Detected
21	Human enterovirus type 71	ATCC	VR-784	Not Detected
22	Human respiratory syncytial virus A	ATCC	VR-26	Not Detected
23	Human respiratory syncytial virus B	ATCC	VR-955	Not Detected
24	Parainfluenza 1 virus	ATCC	VR-1380	Not Detected
25	Human parainfluenza virus 2	ATCC	VR-92	Not Detected

NO.	Organism	Source	Isolate No.	Result†
26	Human parainfluenza virus 3	ATCC	VR-93	Not Detected
27	Human parainfluenza 4 virus 4a	ATCC	VR-1378	Not Detected
28	Human parainfluenza virus 4b	ATCC	VR-1377	Not Detected
29	Human Metapneumovirus (MPV)	KBPV	VR-87	Not Detected
30	Human adenovirus 1	ATCC	VR-1	Not Detected
31	Human adenovirus 11	KBPV	VR-63	Not Detected
32	Human adenovirus 18	ATCC	VR-1095	Not Detected
33	Human adenovirus 23	ATCC	VR-1101	Not Detected
34	Human adenovirus 3	ATCC	VR-3	Not Detected
35	Human adenovirus 4	ATCC	VR-1572	Not Detected
36	Human adenovirus 8	ATCC	VR-1368	Not Detected
37	Human adenovirus type 31	ATCC	VR-1109	Not Detected
38	Human adenovirus type 40	ATCC	VR-931	Not Detected
39	Human adenovirus type 5	KBPV	VR-61	Not Detected
40	Human adenovirus type 35	ATCC	VR-718	Not Detected
41	Human Bocavirus (HBoV)	Korean isolate		Not Detected
42	*Legionella pneumophila* Serotype 2	ATCC	33154	Not Detected
43	*Legionella pneumophila* subsp. fraseri Serotype 4	ATCC	33156	Not Detected
44	*Legionella pneumophila* Serotype 7	ATCC	33823	Not Detected
45	*Legionella pneumophila* Serotype 10	ATCC	43283	Not Detected
46	*Legionella pneumophila* Serotype 11	ATCC	43130	Not Detected
47	*Legionella pneumophila* Serotype 12	ATCC	43290	Not Detected
48	*Legionella pneumophila* Serotype 13	ATCC	43736	Not Detected
49	*Legionella pneumophila* Serotype 14	ATCC	43703	Not Detected
50	*Legionella pneumophila* subsp. fraseri Serotype 15	ATCC	35251	Not Detected
51	*Mycoplasma pneumoniae*	ATCC	15293	Not Detected
52	*Streptococcus salivarius*	KCTC	5512	Not Detected
53	*Staphlococcus epidermidis*	KCCM	40416	Not Detected
54	*Haemophilus influenzae*	ATCC	51907	Not Detected
55	*Mycobacterium tuberculosis*	ATCC	25177	Not Detected

NO.	Organism	Source	Isolate No.	Result†
56	*Alphacoronavirus 1 (feline infectious Peritonitis Virus), 79-1146*	BEI	NR-49097	Not Detected
57	*Porcine Respiratory Coronavirus, ISU-1*	BEI	NR-48572	Not Detected
58	*Chlamydia pneumoniae*	ATCC	53592	Not Detected
59	*Streptococcus pneumoniae*	KCCM	40410	Not Detected
60	*Streptococcus pyrogenes*	ATCC	19615	Not Detected
61	*Bordetella pertussis*	ATCC	BAA-589	Not Detected
62	*Pneumocystis jirovecii* (PJP)	Korean isolate		Not Detected
63	Pooled human nasal wash*	Korean isolate		Not Detected
64	*Candida albicans*	KCCM	11282	Not Detected
65	*Pseudomonas aeruginosa*	ZMC	801908	Not Detected
66	*Corynebacterium diphtheriae*	KCTC	3075	Not Detected
67	*Escherichia coli*	KCTC	2443	Not Detected
68	*Lactobacillus acidophilus*	KCTC	3140	Not Detected
69	*Legionella longbeachae*	KCTC	33462	Not Detected
70	*Moraxella catarrhalis*	KCTC	42706	Not Detected
71	*Neisseria meningitidis*	KCCM	41562	Not Detected
72	Cytomegalovirus	ZMC	0810003CF	Not Detected
73	Epstein-Barr virus	ATCC	VR-1492	Not Detected
74	Human adenovirus 7	KBPV	VR-26	Not Detected
75	*Neisseria elongata subsp. Elongata*	KCTC	23361	Not Detected

† Specificity tests were repeated 3 times.
* No.63 was used to evaluate the specificity of diverse microbial flora in the human respiratory tract.
※ ATCC: American Type Culture Collection,
BEI: BEI Resources
KBPV: Korea Bank for Pathogenic Viruses
ZMC: ZeptoMetrix Corporation
KCTC : Korean Collection for Type Cultures

2. Analytical Sensitivity

In order to determine the sensitivity of Allplex™ SARS-CoV-2 Assay, genomic RNA from SARS-CoV-2, obtained from TWIST BIOSCIENCE (Cat. No. 102024) and BEI Resources (Cat. No. NR-52286) was serially diluted into negative sample matrix. Nucleic acids were extracted from each dilution using Microlab NIMBUS IVD and analyzed with Allplex™ SARS-CoV-2

Assay. Detection limit of Allplex™ SARS-CoV-2 Assay verified using genomic RNA of TWIST BIOSICENCE is 5000 copies/mL (= 50 RNA copies/rxn). Detection limit of Allplex™ SARS-CoV-2 Assay verified using inactivated SARS-CoV-2 virus of BEI Resources is 1,000 viral genome equivalents/mL (= 1.0 GE/μL).

In order to determine the sensitivity of Allplex™ SARS-CoV-2 Assay for swab extraction-free method, inactivated SARS-CoV-2 virus of BEI Resources (Cat. No. NR-52286) was roughly extracted with extraction-free method. Detection limit for extraction-free method is 10,000 viral genome equivalents/mL (= 10 GE/μL).

In order to determine the sensitivity of Allplex™ SARS-CoV-2 Assay for saliva extraction-free method, inactivated SARS-CoV-2 virus of BEI Resources (Cat. No. NR-52286) was roughly extracted with extraction-free method. Detection limit for extraction-free method is 20,000 viral genome equivalents/mL (= 20 GE/μL).

3. Reproducibility

The reproducibility test was prepared including High Negative (0.1X LoD), Low positive (1X LoD) and Moderate positive (3X LoD) samples. At each testing site, the kit was tested for five days, two runs per day by two different experimenters and triplicate of each target. The positive rates were observed for each target for reproducibility study: 100.0% for Moderate positive samples, ≥95% for Low positive samples. The reproducibility of the Allplex™ SARS-CoV-2 Assay was evaluated between runs, sites and product lots. Positive rates for all concentrations and CV values met criteria of less than or equal to 4%(≤4%).

The results were satisfied with the Criteria set above, thus confirming the reproducible performances of Allplex™ SARS-CoV-2 Assay.

4. Interfering substances

There were no effects on the results by adding the substance: non-specific detections or inhibitions on target amplification. Based on the results, 16 interfering substances had no effect on Allplex™ SARS-CoV-2 Assay results.

No.	Interfering Substances	Source	Test Concentration
1	Mucin (bovine submaxillary gland, type I-S)	Sigma-Aldrich (Cat.No.M3895)	60 μg/ml
2	Mupirocin (Antibiotic, nasal ointment)	Sigma-Aldrich (Cat.No.1448901)	6.6 mg/ml
3	Oxymetazoline (Afrin Nasal Spray)	Sigma-Aldrich (Cat.No.O2378)	15% (v/v)
4	Blood	Human	2% (v/v)

5	Tobramycin (Antibacterial, systemic)	Sigma-Aldrich (Cat.No.T4014)	4.0 μg/mL
6	Zanamivir (Anti-viral drug-Relenza)	Sigma-Aldrich (Cat.No.SML0492)	3.3 mg/mL
7	Oseltamivir (Anti-viral drug-Tamiflu)	Sigma-Aldrich (Cat.No.1479304)	25 mg/mL
8	Pulmicort® (Nasal corticosteroid) - Budesonide	AstraZeneca (Cat.No.199403233)	125 μg/mL
9	Benzocaine (Throat lozenges)	Sigma-Aldrich (Cat.No.E1501)	25 mg/mL
10	Menthol (Throat lozenges)	Sigma-Aldrich (Cat.No.M2772)	25 mg/mL
11	Zinc sulfate	Sigma-Aldrich (Cat.No.83265)	0.55 mg/mL
12	Sodium chloride	Sigma-Aldrich (Cat.No.S3014)	0.8% (w/v)
13	Phenylephrine hydrochloride (0.5%)	Sigma-Aldrich (Cat.No. P6126)	15% (v/v)
14*	Nicotine	Sigma-Aldrich (Cat.No.N3876)	0.12 mg/mL
15*	Toothpaste	Colgate®	0.0625% (v/v)
16*	Mouthwash	Listerine®	20% (v/v)

* These interfering substances, which could be found in saliva sample, were tested using saliva specimen only.

5. Clinical performance

5-1. Standard extraction

[Comparison study with CE-IVD approved comparator]

A total of 187 specimens were included in this clinical performance. The specimens consist of 5 nasopharyngeal aspirate samples, 61 nasopharyngeal swab samples, 5 bronchoalveolar lavage samples, 59 oropharyngeal (throat) swab samples, and 57 sputum samples. Clinical performance of the Allplex™ SARS-CoV-2 Assay was evaluated through the comparison with other SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel which is CE-IVD approved before. This comparison test is shown more than 95% rate of agreement in clinical sample. Therefore, it is confirmed that the quality of Allplex™ SARS-CoV-2 Assay is valid. The performance is summarized in table.

		CE-IVD Approved Comparator Result		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 Assay	Positive	96	3*	99
	Negative	0	88	88
	Total	96	91	187

- ORA(Overall rates of agreement): 98.40% (95% CI: 95.38% to 99.67%)

- PPA(Positive Percent Agreement): 100% (95% CI: 96.23% to 100.00%)
- NPA(Negative Percent Agreement): 96.70% (95% CI: 90.67% to 99.31%)

* Sequencing and confirms true positive.

[Comparison between nasopharyngeal swab (NPS) and saliva specimen]

A total of 80 saliva samples were included in this clinical performance of standard extraction for saliva. In order to prove the clinical validity of standard extraction for saliva, it was evaluated by comparing with standard extraction for nasopharyngeal swab. This comparison test showed higher than 95% rate of agreement in clinical sample. Therefore, it is confirmed that the quality of standard extraction for saliva of Allplex™ SARS-CoV-2 Assay is valid. The performance is summarized in table.

Allplex™ SARS-CoV-2 Assay		Standard extraction for NPS		
		Positive	Negative	Total
Standard extraction for Saliva	Positive	30	0	30
	Negative	0	50	50
	Total	30	50	80

- ORA(Overall rates of agreement): 100.00% (95% CI: 95.49% to 100.00%)
- PPA(Positive Percent Agreement): 100.00% (95% CI: 88.43% to 100.00%)
- NPA(Negative Percent Agreement): 100.00% (95% CI: 92.89% to 100.00%)

[Comparison between nasopharyngeal swab (NPS) and combo-swab (nasal swab + oral swab)]

A total of 84 combo-swab (nasal swab + oral swab) samples were included in this clinical performance of standard extraction for combo-swab (nasal swab + oral swab). Two types of samples, nasopharyngeal swab and combo-swab (nasal swab + oral swab), were taken per patient. Each sample was tested with the Allplex™ SARS-CoV-2 Assay by standard extraction. In order to prove the clinical validity of standard extraction for combo-swab (nasal swab + oral swab), it was evaluated by comparing with standard extraction for nasopharyngeal swab. This comparison test showed higher than 95% rate of agreement in clinical sample. Therefore, it is confirmed that the quality of standard extraction for combo-swab (nasal swab + oral swab) of Allplex™ SARS-CoV-2 Assay is valid. The performance is summarized in tables below

Allplex™ SARS-CoV-2 Assay		Standard extraction for NPS		
		Positive	Negative	Total
Standard extraction for combo-swab (nasal swab + oral swab)	Positive	52	0	52
	Negative	2	30	32
	Total	54	30	84

- OPA(Overall Percent Agreement): 97.62% (95% CI: 91.66% to 99.71%)

- PPA(Positive Percent Agreement): 96.30% (95% CI: 87.25% to 99.55%)
- NPA(Negative Percent Agreement): 100% (95% CI: 88.43% to 100.00%)

5-2. Extraction-free for swab

A total of 111 swab specimens were included in this clinical performance of extraction-free method for swab. This study was compared with standard extraction to prove the clinical validity of swab extraction-free method for Allplex™ SARS-CoV-2 Assay. This comparison test is shown more than 95% rate of agreement in clinical sample. Therefore, it is confirmed that the quality of swab extraction-free method for Allplex™ SARS-CoV-2 Assay is valid. The performance is summarized in table.

Allplex™ SARS-CoV-2 Assay		Standard Extraction		
		Positive	Negative	Total
Extraction-free for swab	Positive	78	0	78
	Negative	1*	32	33
	Total	79	32	111

- ORA(Overall rates of agreement): 99.1% (95% CI: 95.07% to 99.8%)
- PPA(Positive Percent Agreement): 98.7% (95% CI: 93.17 % to 99.78%)
- NPA(Negative Percent Agreement): 100% (95% CI: 89.57% to 100%)

* Sequencing and confirms true positive.

5-3. Extraction-free for saliva

Paired nasopharyngeal swab and saliva specimens were collected from 80 patients. A total of 160 nasopharyngeal swab and saliva specimens were included in this clinical performance of extraction-free method for saliva. This study was compared with extraction-free method for swab to prove the clinical validity of saliva extraction-free method for Allplex™ SARS-CoV-2 Assay. This comparison test is shown more than 95% rate of agreement in clinical sample. Therefore, it is confirmed that the quality of saliva extraction-free method for Allplex™ SARS-CoV-2 Assay is valid. The performance is summarized in table.

Allplex™ SARS-CoV-2 Assay		Extraction-free for swab		
		Positive	Negative	Total
Extraction-free for saliva	Positive	30	0	30
	Negative	0	50	50
	Total	30	50	80

- ORA(Overall rates of agreement): 100.00% (95% CI: 95.49% to 100.00%)
- PPA(Positive Percent Agreement): 100.00% (95% CI: 88.43% to 100.00%)
- NPA(Negative Percent Agreement): 100.00% (95% CI: 92.89% to 100.00%)

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SYMBOLS

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
PREMIX	PCR Master Mix or Detection Mix
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
UDI	Unique Device Identifier
rxns	Reaction barcode for automated extraction system

ORDERING INFORMATION

Cat. No.	Product	Size
Allplex™ series		
RV10247Y	Allplex™ SARS-CoV-2 Assay	50 rxns
RV10248X	**Allplex™ SARS-CoV-2 Assay**	**100 rxns***

* For use with the Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, and Seegene STARlet only

Accessory product		
SG1701	Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	50 preps
Automated extraction systems		
65415-02	Microlab NIMBUS IVD	EA
173000-075	Microlab STARlet IVD	EA
65415-03	Seegene NIMBUS	EA
67930-03	Seegene STARlet	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384 T / 1box
EX00013C	STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 T / 1box
SGprep32-180701	SGprep32	EA
EX00003P	STARMag 96 UniPlate	96 T / 1box
EX00004T	STARMag 96 UniTube	96 T / 1box
SG71100	SEEPREP32	EA
EX00009P	STARMag 96 ProPrep (Plate Type)	96 T / 1box
EX00009T	STARMag 96 ProPrep (Tube Type)	96 T / 1box
EX00017P	STARMag 96 ProPrep C (Plate Type)	96 T / 1 box
EX00017T	STARMag 96 ProPrep C (Tube Type)	96 T / 1 box
M9600	Maelstrom™ 9600	EA
W665S66	TANBead® Nucleic Acid Extraction Kit OptiPure Viral Auto Tube	72 T / 1box
W665A10	TANBead® Nucleic Acid Extraction Kit OptiPure Viral Bulk Plate	960 T / crt

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress CoV-2/Flu/RSV *plus*

REF **XP3COV2/FLU/RSV-10**

Instructions for Use

For Use with GeneXpert® Dx or GeneXpert® Infinity Systems

IVD

Cepheid

For *In Vitro* Diagnostic Use

302-8927, Rev. A
July 2022

Trademark, Patents and Copyright Statements

See Section 24, Revision History for a description of changes.

Xpert® Xpress CoV-2/Flu/RSV *plus*

1 Proprietary Name

Xpert® Xpress CoV-2/Flu/RSV *plus*

2 Common or Usual Name

Xpert Xpress CoV-2/Flu/RSV *plus*

3 Intended Use

The Xpert Xpress CoV-2/Flu/RSV *plus* test is a rapid, multiplexed real-time RT-PCR test intended for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, influenza A, influenza B, and/or respiratory syncytial virus (RSV) in either nasopharyngeal swab or anterior nasal swab specimens collected from individuals suspected of respiratory viral infection, consistent with COVID-19, by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus and RSV nucleic acids in clinical specimens and is not intended to detect influenza C virus. The SARS-CoV-2, influenza A, influenza B and RSV RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

Testing with the Xpert Xpress CoV-2/Flu/RSV *plus* test is intended for use by trained operators who are proficient in performing tests using GeneXpert Dx and/or GeneXpert Infinity systems.

The Xpert Xpress CoV-2/Flu/RSV *plus* test is intended to be performed by trained users in laboratory settings.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has since spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily via aerosolized droplets (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[3,4]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.[5] Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, the elderly, and those who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[6] The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[6] By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected.[6] RSV remains the leading cause for hospitalizations in infants worldwide.[7] Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season usually mirrors influenza as infections begin to rise during the fall and last through early spring.[5,6]

SARS-CoV-2, influenza, and RSV viruses can cause infections that present with very similar symptoms, making clinical differentiation between them very difficult.[8] Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza and RSV. The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

5 Principle of the Procedure

The Xpert Xpress CoV-2/Flu/RSV *plus* test is an automated *in vitro* diagnostic test for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, Flu A, Flu B, and RSV. The Xpert Xpress CoV-2/Flu/RSV *plus* test is performed on GeneXpert Instrument Systems (Dx and Infinity Systems). The primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test are designed to amplify and detect unique sequences in the following: nucleocapsid (N) and envelope (E) and RNA-dependent RNA polymerase (RdRP) genes of the SARS-CoV-2 virus genome, influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress CoV-2/Flu/RSV *plus* test includes reagents for the detection of SARS-CoV-2, Flu A, Flu B and RSV viral RNA in either nasopharyngeal swab or anterior nasal swab specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or 2 mL of eNAT™. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress CoV-2/Flu/RSV *plus* kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress CoV-2/Flu/RSV *plus* Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Lysis Reagent	1.0 mL per cartridge
• Binding Reagent	1.0 mL per cartridge
• Elution Reagent	3.0 mL per cartridge
• Wash Reagent	0.4 mL per cartridge
Disposable Transfer Pipettes	**10-12 per kit**
Flyer	**1 per kit**

- Instructions to locate (and import) the ADF and EUA documentation such as the Product Insert on www.cepheid.com.

Quick Reference Instructions	**2 per kit**

(For use with the GeneXpert Xpress System only)

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress CoV-2/Flu/RSV *plus* cartridges at 2–28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

  For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

  For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from Microbiologics, Inc. (St. Cloud, MN).

- 8246 Flu/RSV/SARS-CoV-2 Control Panel (Inactivated Swab)

eNAT Molecular Collection and Preservation Medium from Copan Italy S.p.A. (Brescia, IT):

- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U073S01
- eNAT Molecular Collection and Preservation Medium, Copan Catalog #6U074S01

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of Flu A, Flu B, RSV, and/or SARS-CoV-2 RNA.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[9] and the Clinical and Laboratory Standards Institute.[10]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Refer to Copan eNAT® Package Insert for safety and handling information.
- Avoid direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids and bases. These mixtures could release noxious gas.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Xpress CoV-2/Flu/RSV *plus* cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution's standard procedures for a contamination or spill event. For equipment, follow the manufacturer's recommendations for decontamination of equipment.

11 Chemical Hazards[11, 12]

- **Signal Word: Warning**
- **UN GHS Hazard Statements**
  - Harmful if swallowed
  - May be harmful in contact with skin
  - Causes eye irritation
- **UN GHS Precautionary Statements**
  - **Prevention**
    - Wash hands thoroughly after handling.
  - **Response**
    - Call a POISON CENTER or doctor/physician if you feel unwell.
    - If skin irritation occurs: Get medical advice/attention.
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
    - If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure and Section 12.2 for nasal swab collection procedure. Nasopharyngeal and nasal swab specimens can be stored at room temperature (15–30 °C) for up to 48 hours in viral transport medium or eNAT until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal and nasal swab specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium and up to six days in eNAT until testing is performed on the GeneXpert Instrument Systems.

Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19)

https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

Refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19).

https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

12.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1). Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

Figure 1. Nasopharyngeal Swab Collection

12.2 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

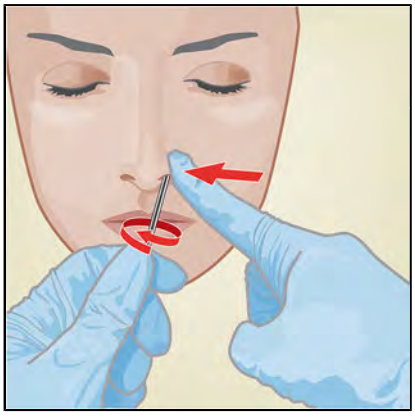


Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

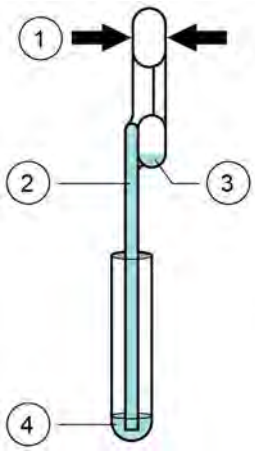
3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 2 mL of eNAT. Break swab at the indicated break line and cap the specimen collection tube tightly.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 4).



Number	Description
1	Squeeze here
2	Pipette
3	Overflow Reservoir Bulb
4	Sample

Figure 4. Transfer Pipette

7. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 4). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) of the cartridge shown in Figure 5. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

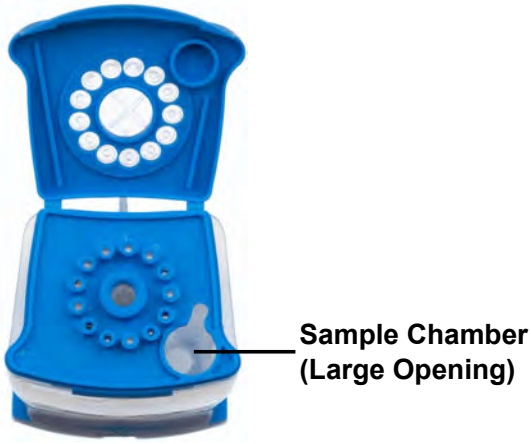


Figure 5. Xpert Xpress CoV-2/Flu/RSV *plus* Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample volume is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress CoV-2/Flu/RSV *plus* test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress CoV-2/Flu/RSV *plus* Assay Definition File (ADF) is imported into the software.

This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - **GeneXpert Dx:**

     If using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

     or
   - **GeneXpert Infinity System:**

     If using the GeneXpert Infinity instrument, power up the instrument by turning the power switch clockwise to the **ON** position. On the Windows desktop, double-click the Xpertise Software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the GeneXpert System window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).

4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date, and Selected Assay.

Note If the barcode on the Xpert Xpress CoV-2/Flu/RSV *plus* cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or **Submit** (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

   For the GeneXpert Dx Instrument:

   a. Locate the module with the blinking green light, open the instrument module door and load the cartridge.
   b. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.
   c. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

   or

   For the GeneXpert Infinity System:

   a. After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click OK to continue. The cartridge will be automatically loaded, the test will run, and the used cartridge will be placed onto the waste shelf for disposal.
   b. When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress CoV-2/Flu/RSV *plus* test provides test results based on the detection of respective gene targets according to the algorithms.

The format of the test results presented will vary depending on the user's choice to run either an Xpress SARS-CoV-2_Flu_RSV plus, Xpress SARS-CoV-2_Flu plus or Xpress SARS-CoV-2 plus test.

Table 1 shows the possible result outcomes when the Xpress SARS-CoV-2_Flu_RSV plus test mode is selected.

Table 1. Xpress SARS-CoV-2_Flu_RSV plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
RSV POSITIVE	The RSV target RNA is detected. ● The RSV signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. ● SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting ● Probe Check: PASS; all probe check results pass

Result	Interpretation
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1]If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another test, if coinfection would change clinical management.

Table 2 shows the possible result outcomes when the Xpress SARS-CoV-2_Flu plus test mode is selected.

Table 2. Xpress SARS-CoV-2_Flu plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
Flu A POSITIVE	The Flu A target RNA is detected. ● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
Flu B POSITIVE	The Flu B target RNA is detected. ● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: Pass; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected. ● SARS-CoV-2, Flu A, and Flu B target RNAs are not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting. ● Probe Check: PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Result	Interpretation
NO RESULT	Presence or absence of SARS-CoV-2, Flu A and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another test, if coinfection would change clinical management.

Table 3 shows the possible result outcomes when the Xpress SARS-CoV-2 plus test mode is selected.

Table 3. Xpress SARS-CoV-2 plus Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred ● Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target RNA is not detected. ● SARS-CoV-2 target RNA is not detected ● SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting ● Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting ● Probe Check: PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. ● SPC: NO RESULT ● Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2 of the IFU. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

The Xpert Xpress CoV-2/Flu/RSV *plus* test can be run to detect SARS-CoV-2, Flu and RSV by selecting Xpress SARS-CoV-2_Flu_RSV plus from the Select Test menu; SARS-CoV-2 and Flu only by selecting Xpress SARS-CoV-2_Flu plus; or SARS-CoV-2 only by selecting Xpress SARS-CoV-2 plus. The Xpress SARS-CoV-2 plus test mode includes an Early Assay Termination (EAT) function that will provide earlier time to result in high titer specimens if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen, and its results may not be reported.

17 Retests

17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid Technical Support for assistance.

17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress CoV-2/Flu/RSV *plus* cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly inverting the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

18 Limitations

- Performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test has only been established in nasopharyngeal swab and anterior nasal swab specimens. Use of the Xpert Xpress CoV-2/Flu/RSV *plus* test with other specimen types has not been assessed and performance characteristics are unknown.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- As with any molecular test, mutations within the target regions of the Xpert Xpress CoV-2/Flu/RSV *plus* test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress CoV-2/Flu/RSV *plus* test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist *in vivo*, independent of virus infectivity. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for patients without signs and symptoms of respiratory tract infection.
- This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza, or RSV.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Results from analytical studies with contrived co-infected samples showed potential for competitive interference of influenza B or RSV A at low concentrations (~3X LoD) when influenza A concentration is >1.7e5 RNA copies/mL or 1.7e6 RNA copies/mL, respectively. In addition, there is potential for competitive interference of influenza B at low concentration (~3X LoD) when SARS-CoV-2 concentration is >1e5 RNA copies/mL.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- Zicam at 15% (w/v) may interfere with the detection of low levels of influenza B and RSV A.
- As the Xpert Xpress CoV-2/Flu/RSV *plus* test does not differentiate between the N2, RdRP and E gene targets, the presence of other coronaviruses in the B lineage, Betacoronavirus genus, including SARS-CoV may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Performance has not been established with media containing guanidine thiocyanate (GTC) other than eNAT.

19 Performance Characteristics

19.1 Clinical Evaluation

The performance of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated using archived clinical nasopharyngeal (NP) swab specimens in viral transport medium or universal transport medium. Archived specimens were selected consecutively by date and previously known analyte result. A total of 279 NP swab specimens were tested with Xpert Xpress CoV-2/Flu/RSV *plus* side by side with another SARS-CoV-2 RT-PCR test included in ARTG and another influenza/RSV molecular test included in ARTG in a randomized and blinded fashion.

Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and non-determinate rate were determined by comparing the results of the Xpert Xpress CoV-2/Flu/RSV *plus* test relative to the results of another SARS-CoV-2 RT-PCR test included in ARTG for the SARS-CoV-2 target, and another influenza/RSV molecular test included in ARTG for the Flu A, Flu B, and RSV targets, respectively.

Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 1.4% (4/279). On repeat testing, all four (4) specimens yielded valid results. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.0% (0/279).

Table 4. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	279	66	0	213	0	100.0% (94.5% - 100.0%)	100.0% (98.2% - 100.0%)
Flu A	264	51	0	213	0	100.0% (93.0% - 100.0%)	100.0% (98.2% - 100.0%)
Flu B	264	46	0	218	0	100.0% (92.3% - 100.0%)	100.0% (98.3% - 100.0%)
RSV	264	47	0	217	0	100.0% (92.4% - 100.0%)	100.0% (98.3% - 100.0%)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

For the NS specimens, Xpert Xpress CoV-2/Flu/RSV *plus* demonstrated a PPA and NPA of 100.0% and 100.0% for SARS-CoV-2, respectively; 100.0% and 99.5% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 5). The initial non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 2.1% (5/240). Four (4) of the five (5) specimens gave valid results upon retest. One specimen was not re-tested due to insufficient volume. The final non-determinate rate for the Xpert Xpress CoV-2/Flu/RSV *plus* test was 0.4% (1/240).

Table 5. Xpert Xpress CoV-2/Flu/RSV *plus* Performance Results Using NS Specimens

Target	Number of Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
Flu A	239	48	1	191	0	100.0% (92.6% - 100.0%)	99.5% (97.1% - 99.9%)
Flu B	239	48	0	191	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
RSV	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval							

19.2 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was first estimated using two reagent lots by testing limiting dilutions of seven respiratory viruses (NATtrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B Victoria lineage, Flu B Yamagata lineage, RSV A and RSV B) into pooled negative clinical NP swab matrix, following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress CoV-2/Flu/RSV *plus* reagents. The verified LoD values for the viruses tested are summarized in Table 6.

Table 6. Xpert Xpress CoV-2/Flu/RSV *plus* Limit of Detection

Virus/Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	138 copies/mL
Influenza A/Idaho/07/2018	0.007 $TCID_{50}$/mL
Influenza A/Hong Kong/45/2019	0.44 FFU/mL
Influenza B/Washington/2/2019	12.9 $CEID_{50}$/mL
Influenza B/Wisconsin/10/2016	2.4 $TCID_{50}$/mL
RSV A/2/Australia/61	0.33 $TCID_{50}$/mL
RSV B/9320/MA/77	0.37 $TCID_{50}$/mL

19.3 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress CoV-2/Flu/RSV *plus* was evaluated on September 27, 2021 using *in silico* analysis of the assay amplicons in relation to 2,685,478 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E, N2 and RdRP.

For analysis of the E target, 3,818 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,681,660 sequences. Of the 2,681,660 GISAID sequences, 2,667,594 (99.48%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 13,990 sequences and two mismatches or more were observed for 76 sequences. Of the 76 sequences with two or more mismatches, 43 sequences contained 2 or 3 mismatches in the forward primer region; one sequence contained 3 mismatches in the reverse primer region; and one sequence contained 2 mismatches in the forward primer and 2 mismatches in the reverse primer. These double and triple mismatches could have a negative impact on the performance of the assay.

For analysis of the N2 target, 4,110 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 2,681,368 sequences. Of the 2,681,368 GISAID sequences, 2,608,487 (97.3%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches

were observed for 70,212 sequences. Two or three mismatches were observed for 2,669 sequences. Of the 31 sequences with three variant positions, 5 sequences have two of the mismatched nucleotides in the probe region and 5 of the sequences have two of the mismatched nucleotides in the reverse primer region. These double mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The RdRP is amplified using a semi-nested primer/probe set; only the inner amplicon is used for the *in silico* analysis. For analysis of the RdRP target, 1,374 sequences were excluded due to ambiguous nucleotides, which reduced the total to 2,684,104 sequences. Of the 2,684,104 GISAID sequences, 2,657,136 (99.0%) were an exact match to the SARS-CoV-2 RdRP target amplicon generated in the Xpert Xpress CoV-2/Flu/RSV *plus* test. Single nucleotide mismatches were observed for 26,864 sequences and two or more mismatches were observed for 77 sequences. Two sequences have 5 mismatches, three located in the probe region and two in the reverse primer region; 20 sequences have two nucleotide mismatches in the forward primer or probe region. These mismatches could have an impact on probe or reverse primer binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for inclusivity, the inclusivity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench testing against multiple strains of SARS-CoV-2, influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 84 strains comprised of 5 SARS-CoV-2 virus strains, 4 SARS-CoV-2 in vitro RNA transcripts representing variant strains, 69 influenza viruses (48 influenza A and 21 influenza B) and 6 RSV strains (4 RSVA and 2 RSV B) were tested in this study with the Xpert Xpress CoV-2/Flu/RSV *plus* test. Three replicates were tested for each strain. All SARS-CoV-2, Flu and RSV strains tested positive in all three replicates. Results are shown in Table 7.

Table 7. Analytical Reactivity (Inclusivity) of the Xpert Xpress CoV-2/Flu/RSV *plus* Test

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
SARS-CoV-2	NATtrol SARS-CoV-2 USA-WA1/2020	412 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Hong Kong/VM20001061/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/Italy-INMI1	4 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/South_Africa/ KRISP-K005325/2020	0.2 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2/England/ 204820464/2020	0.5 $TCID_{50}$/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA USA/WA2/2020(C09)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2RNA/ England/205041766/ 2020(C14)[a]	100 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /England/ MILK-9E05B3/2020 (C15)[a]	200 copies/mL	POS	NEG	NEG	NEG
	SARS-CoV-2 RNA /Japan (Brazil)/IC-0564/2021 (C17)[a]	100 copies/mL	POS	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/WS/33	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/PR/8/34	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Mal/302/54	0.156 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Denver/1/57	10 $CEID_{50}$/mL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/New Jersey/8/76	5.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/New Caledonia/20/1999	0.10 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/New York/55/2004	30 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Solomon Island/3/2006	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Taiwan/42/06	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/59/2007	0.060 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Swine/NY/02/2009	20 $TCID_{50}$/mL	NEG	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/Colorado/14/2012	0.13 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/45/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Iowa/53/2015	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Michigan/272/2017	1.0 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Idaho/07/2018	0.0159 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/505/2018	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/66/2019	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/02/2020	NA[b]	NEG	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Port Chalmers/1/73	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Hawaii/15/2001	100 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Wisconsin/67/05[c]	0.22 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Brisbane/10/2007	0.025 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Minnesota/11/2010	30 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Indiana/08/2011	0.25 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/50/2012	0.050 $TCID_{50}$/mL	NEG	POS	NEG	NEG
	A/Alaska/232/2015	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Singapore/ INFIMH-16-0019/2016	20 $CEID_{50}$/mL	NEG	POS	NEG	NEG
	A/Texas/71/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Kansas/14/2017	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Wisconsin/04/2018	1.0 FFU/mL	NEG	POS	NEG	NEG
	A/Arizona/45/2018	2.0 FFU/mL	NEG	POS	NEG	NEG
	A/Hong Kong/45/2019	2.0 FFU/mL	NEG	POS	NEG	NEG
Avian influenza A[d]	A/Mallard/NY/6750/78 (H2N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/duck/Hunan/ 795/2002 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
	A/Vietnam/1194/2004 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	<1 pg/µL	NEG	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/duck/LTC-10-82743 (H7N2)	<1 pg/µL	NEG	POS	NEG	NEG
	A/chicken/New Jersey/15086/3 (H7N3)	<1 pg/µL	NEG	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	0.612 ng/µL	NEG	POS	NEG	NEG
	A/Shanghai/1/2013 (H7N9)	NA[e]	NEG	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/1996 (H9N2)	<1 pg/µL	NEG	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 PFU/mL	NEG	NEG	POS	NEG
	B/Allen/45	0.25 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/GL/1739/54	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/1/59	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $CEID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Victoria Lineage	B/Panama/45/90	1.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Malaysia/2506/04	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/02/06	0.025 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Brisbane/60/2008	0.05 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Maryland/15/2016	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Colorado/6/2017	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Hawaii/01/2018	8.0 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Missouri/12/2018(NA D197E)	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Washington/02/2019	60 $TCID_{50}$/mL	NEG	NEG	POS	NEG
Influenza B Yamagata Lineage	B/Florida/07/2004	0.50 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Florida/04/06	0.25 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/01/2010	0.50 $CEID_{50}$/mL	NEG	NEG	POS	NEG
	B/Wisconsin/10/2016	20 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Indiana/17/2017	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG
	B/Oklahoma/10/2018	10 $TCID_{50}$/mL	NEG	NEG	POS	NEG

Virus	Strain	Tested Titer	SARS-CoV-2	Flu A	Flu B	RSV
RSV A	RSV-A/NY	0.386 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI-629.8.2/2007	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A/WI/629-11-1_2008	0.50 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-A, Strain: 4/2015 Isolate #1	0.25 $TCID_{50}$/mL	NEG	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS
	RSV-B-CH93(18)-18-01	0.10 $TCID_{50}$/mL	NEG	NEG	NEG	POS

[a] *in vitro* RNA transcripts

[b] Titer A/Indiana/02/2020 virus was without titer and was diluted 100,000-fold in simulated background matrix for testing.

[c] One of three replicates reported ERROR. The run was successfully repeated to obtain three valid replicates.

[d] Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.

[e] Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000-fold in simulated background matrix and tested due to biosafety regulations.

19.4 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 8 was conducted by mapping the SARS-CoV-2 primers and probes in the Xpert Xpress CoV-2/Flu/RSV *plus* test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 8 is expected based on the *in silico* analysis.

Table 8. Microorganisms Analyzed in the *in silico* Analysis for the SARS-CoV-2 Target

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae

Microorganisms from the Same Genetic Family	High Priority Organisms
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-*pneumophila*
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongata and *N. meningitidis*
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

In addition to the *in silico* analysis of the SARS-CoV-2 primers and probes for cross-reactivity, the analytical specificity of the Xpert Xpress CoV-2/Flu/RSV *plus* test was evaluated by bench-testing a panel of 48 microorganisms comprising 4 human coronaviruses, 1 MERS coronavirus and 43 common respiratory pathogens or those potentially encountered in the nasopharynx. The panel was tested in different pools of microorganisms; if a pool produced a positive result, then each member of the pool would have been tested individually. Three replicates of each pool were tested. A sample was considered negative if all three replicates were negative. The bacterial and yeast strains were tested at concentrations of $\geq 1 \times 10^6$ CFU/mL with the exception of *Chlamydia pneumoniae* which was tested at 1.2×10^6 IFU/mL and *Lactobacillus reuteri* which was tested at 5×10^7 copies/mL of genomic DNA. Viruses were tested at concentrations of $\geq 1 \times 10^5$ $TCID_{50}$/mL. The analytical specificity was 100%. Results are shown in Table 9.

Table 9. Respiratory Microorganisms and Human Coronavirus Tested, Concentrations and Xpert Xpress CoV-2/Flu/RSV *plus* Test Results

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Negative Control	NA	NEG	NEG	NEG	NEG
Positive Control	NA	POS	POS	POS	POS
Human coronavirus NL63	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
MERS-coronavirus	1.17e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus 229E	1.21e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus OC43	1.02e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human coronavirus HKU1	1.23e6 copies/mL	NEG	NEG	NEG	NEG
Adenovirus Type 1	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Adenovirus Type 7	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Cytomegalovirus	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Echovirus	1.14e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Enterovirus	2.80e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Epstein Barr Virus	5.60e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
HSV	1.97e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human metapneumovirus	4.07e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 1	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 2	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 3	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Human parainfluenza Type 4	1.19e6 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Measles	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Mumps virus	1.2e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Rhinovirus Type 1A	1.0e5 $TCID_{50}$/mL	NEG	NEG	NEG	NEG
Acinetobacter baumannii	1.30e7 CFU/mL	NEG	NEG	NEG	NEG
Bordetella pertussis	6.40e7 CFU/mL	NEG	NEG	NEG	NEG
Burkholderia cepacia	1.90e8 CFU/mL	NEG	NEG	NEG	NEG
Candida albicans	6.30e6 CFU/mL	NEG	NEG	NEG	NEG
Candida parapsilosis	1.45e6 CFU/mL	NEG	NEG	NEG	NEG
Citrobacter freundii	1.73e8 CFU/mL	NEG	NEG	NEG	NEG
Corynebacterium sp.	1.27e7 CFU/mL	NEG	NEG	NEG	NEG
Enterococcus faecalis	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Escherichia coli	1.55e8 CFU/mL	NEG	NEG	NEG	NEG
Hemophilus influenzae	6.62e6 CFU/mL	NEG	NEG	NEG	NEG
Lactobacillus reuteri	5.0e7 copies/mL	NEG	NEG	NEG	NEG
Legionella spp.	1.42e8 CFU/mL	NEG	NEG	NEG	NEG
Moraxella catarrhalis	2.46e6 CFU/mL	NEG	NEG	NEG	NEG
Mycoplasma pneumoniae	2.7e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria meningitides	4.2e6 CFU/mL	NEG	NEG	NEG	NEG
Neisseria mucosa	1.0e8 CFU/mL	NEG	NEG	NEG	NEG
Propionibacterium acnes	8.25e7 CFU/mL	NEG	NEG	NEG	NEG
Pseudomonas aeruginosa	1.05e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus haemolyticus	2.66e6 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus aureus	5.87e7 CFU/mL	NEG	NEG	NEG	NEG
Staphylococcus epidermidis	2.47e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus agalactiae	1.75e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pneumoniae	2.26e7 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus pyogenes	9.0e6 CFU/mL	NEG	NEG	NEG	NEG

Strain	Tested Concentration	SARS-CoV-2	Flu A	Flu B	RSV
Streptococcus salivarius	4.19e6 CFU/mL	NEG	NEG	NEG	NEG
Streptococcus sanguinis	8.67e6 CFU/mL	NEG	NEG	NEG	NEG
Chlamydia pneumoniae	1.20e6 CFU/mL	NEG	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.20e6 CFU/mL	NEG	NEG	NEG	NEG

19.5 Microbial Interference

Microbial interference of the Xpert Xpress CoV-2/Flu/RSV *plus* test caused by the presence of bacterial or viral strains that might be encountered in human upper respiratory tract specimens, was evaluated by testing a panel of 10 commensal microorganisms, consisting of 7 viral strains and 3 bacterial strains. Contrived samples consisted of SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B viruses seeded at 3x the Limit of Detection (LoD) into simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix in the presence of Adenovirus Type 1C, Human Coronavirus OC43, Rhinovirus Type 1A, Human metapneumovirus, Human parainfluenza Types 1, 2, and 3 (each seeded at $1x10^5$ units/mL), *Hemophilus influenzae* (seeded at $1x10^6$ CFU/mL), *Staphylococcus aureus* or *Staphylococcus epidermidis* (each seeded at $1x10^7$ CFU/mL).

Replicates of 8 positive samples were tested for each target virus (SARS-CoV-2, Flu A, Flu B, RSV A, or RSV B) and each potential microbial interference strain combination. For each target, all 8 of 8 replicate samples were correctly identified using the Xpert Xpress CoV-2/Flu/RSV *plus* test. No interference by the commensal viral or bacterial strains was reported.

19.6 Competitive Interference

Competitive interference of the Xpert Xpress CoV-2/Flu/RSV *plus* caused by co-infections were evaluated by testing contrived samples of individual SARS-CoV-2, Flu A, Flu B or RSV strains at 3X LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at 3X LoD was 414 copies/mL for SARS-CoV-2 (inactivated USA-WA1/2020); 0.021 $TCID_{50}$/mL for Flu A/Idaho/072018, 38.7 $CEID_{50}$/mL for Flu B/Washington/2/2019; 0.99 $TCID_{50}$/mL for RSV A/2/Australia/61), and 1.11 $TCID_{50}$/mL for RSV B/9320/MA/77. The competitive strains were evaluated at 10^5 or higher titer units (copies/mL, $TCID_{50}$/mL, $CEID_{50}$/mL or PFU/mL). The corresponding concentration of RNA (copies/mL) for the Flu and RSV strains was determined by droplet digital PCR (ddPCR). Replicates of 3 were tested for each target strain and each competitive strain combination. The virus at high concentration shows no competitive inhibitory effects if 3 of 3 replicates for the target strain report positive results. If the results reported less than 3 of 3 positive replicates, the concentration of the competing virus was reduced by 10-fold increments until no interference was observed. Below is a summary of the results:

Table 10. Summary of Competitive Interference Study with Flu A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)			
		at 1.7e8 RNA copies/mL	at 1.7e7 RNA copies/mL	at 1.7e6 RNA copies/mL	at 1.7e5 RNA copies/mL
Flu B	Flu A	0/3	0/3	2/3	3/3
RSV A		0/3	0/3	3/3	Not tested
RSV B		3/3	Not tested	Not tested	Not tested
SARS-CoV-2		3/3	Not tested	Not tested	Not tested

Table 11. Summary of Competitive Interference Study with Flu B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.4e5 RNA copies/mL
Flu A	Flu B	3/3
RSV A		3/3
RSV B		3/3
SARS-CoV-2		3/3

Table 12. Summary of Competitive Interference Study with RSV A at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 4.6e6 RNA copies/mL
Flu A	RSV A	3/3
Flu B		3/3
SARS-CoV-2		3/3

Table 13. Summary of Competitive Interference Study with RSV B at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3) at 1.9e5 RNA copies/mL
Flu A	RSV B	3/3
Flu B		3/3
SARS-CoV-2		3/3

Table 14. Summary of Competitive Interference Study with SARS-CoV-2 at High Concentration

Test Viruses at 3X LoD	Interferent Virus	Correct Calls (n/3)	
		at 1e6 RNA copies/mL	at 1e5 RNA copies/mL
Flu A	SARS-CoV-2	3/3	Not tested
Flu B		1/3	3/3
RSV A		3/3	Not tested
RSV B		3/3	Not tested

The study showed that Flu A/Idaho/07/2018 at concentrations above 1.7e5 RNA copies/mL inhibited detection of Flu B at 3X LoD, and at concentrations above 1.7e6 RNA copies/mL inhibited detection of RSV A at 3X LoD (Table 10). In addition, SARS-CoV-2 at concentrations above 1e5 RNA copies/mL inhibited detection of Flu B at 3X LoD (Table 14). No other competitive interference was observed for the potential co-infections tested in the study at the concentrations tested.

19.7 Potentially Interfering Substances

Substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and potentially interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with direct testing on the Xpert Xpress CoV-2/Flu/RSV *plus*.

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasopharyngeal swab (NPS)/ nasal swab (NS) matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the LoD determined for each strain. Positive samples tested with the Xpert Xpress CoV-2/Flu/RSV *plus* included one SARS-CoV-2, one influenza A H1N1, one influenza A H3N2, one influenza B and two RSV (RSV A and RSV B) strains. The substances, with active ingredients, that were evaluated are listed in Table 15.

Table 15. Potentially Interfering Substances Tested

Substance ID	Substance/Class	Substance/Active Ingredient
No substance	Control	Copan Universal Transport Medium (UTM)
Albuterol Sulfate	Beta-adrenergic bronchodilator	Albuterol Sulfate (5mg/mL)
Afrin	Nasal Spray	Oxymetazoline, 0.05%
BD Universal Transport Medium	Transport Media	N/A
Copan 3U045N.PH (Cepheid Swab/M)	Transport Media	N/A
Blood	Blood	Blood (Human)
Fluticasone Propionate Nasal Spray	Nasal corticosteroid	Fluticasone Propionate
Menthol	Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol
Mucin	Mucin	Purified Mucin protein (Bovine or porcine submaxillary gland)
Mupirocin	Antibiotic, nasal ointment	Mupirocin (20 mg/g=2%)
PHNY	Nasal Drops	Phenylephrine, 1%
Saline	Saline Nasal Spray	Sodium Chloride (0.65%)
Remel M4RT	Transport Media	N/A
Remel M5	Transport Media	N/A
Tamiflu	Anti-viral drugs	Zanamivir
Tobramycin	Antibacterial, systemic	Tobramycin
Zicam	Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur (0.05%)
Zinc	Zinc supplement	Zinc Gluconate

The results from the study (Table 16) show that for most cases, 8 out of 8 replicates reported positive results for each combination of virus and substance tested and no interference was observed. When Zicam was initially tested at 15% w/v, interference was observed in the detection of Flu B and RSV A. However, when Zicam was tested at 7.5% w/v, no interference was observed.

Table 16. Mean Ct values for Xpert Xpress CoV-2/Flu/RSV *plus* Targets Tested in the Presence of Potentially Interfering Substances

Substance	Concentration Tested	Number of Correct Results/Number Tested					
		SARS-CoV-2/ USA-WA-1	Influenza A/Idaho/07/ 2018	H3N2 Flu A/ Hong Kong/ 45/2019	Flu B/ Washington /02/2019	RSV A/2/ Australia/61	RSV B/9320/ MA/77
Control Simulated NPS/NS Matrix (No substance)	100% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Afrin	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Albuterol Sulfate	0.83 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
BD Universal Transport Medium	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Blood	2% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Copan Swab M	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Fluticasone Propionate Nasal Spray	5 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Menthol	1.7 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Mucin	0.1% (w/v)	8/8	8/8	8/8	8/8	8/8	8/8
Mupirocin	10 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
PHNY	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Remel M4RT	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Remel M5	N/A	8/8	8/8	8/8	8/8	8/8	8/8
Saline	15% (v/v)	8/8	8/8	8/8	8/8	8/8	8/8
Tamiflu	7.5 mg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Tobramycin	4 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8
Zicam	15% (w/v)	8/8	8/8	8/8	5/8[a]	7/8[b]	8/8
Zinc	0.1 µg/mL	8/8	8/8	8/8	8/8	8/8	8/8

[a] With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no clinically significant difference was observed between the control mean Ct and the test mean Ct.

[b] With 15% (w/v) Zicam, a statistically significant difference was observed between the control mean Ct and the test mean Ct. Testing was repeated with 7.5% (w/v) Zicam and no statistically significant difference was observed between the control mean Ct and the test mean Ct.

19.8 Carry-over Contamination

A study was conducted to assess whether the single-use, self-contained Xpert Xpress CoV-2/Flu/RSV *plus* cartridge prevents specimen and amplicon carryover by testing a negative sample immediately after testing of a very high positive sample in the same GeneXpert module. The negative sample used in this study consisted of simulated NPS/NS matrix and the positive sample consisted of high Flu B and high SARS-CoV-2 virus concentrations (Flu B/Wisconsin/10/2016 at 1.0e6 $TCID_{50}$/mL and inactivated SARS-CoV-2 USA-WA1/2020 at 1e4 copies/mL) seeded into negative NPS/NS matrix. The negative sample was tested in a GeneXpert module at the start of the study. Following the initial testing of the negative sample, the high positive sample was processed in the same GeneXpert module immediately followed by another negative sample. This was repeated 20 times in the same module, resulting in 20 positives and 21 negatives for the module. The study was repeated using a second GeneXpert module for a total of 40 positive and 42 negative samples. All 40 positive samples were correctly reported as **SARS-CoV-2 POSITIVE**; **Flu A NEGATIVE**; **Flu B POSITIVE**; **RSV NEGATIVE**. All 42 negative samples were correctly reported as **SARS-CoV-2 NEGATIVE**; **Flu A NEGATIVE**; **Flu B NEGATIVE**; **RSV NEGATIVE** with the Xpert Xpress CoV-2/Flu/RSV *plus* test. No specimen or amplicon carry-over contamination was observed in this study.

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21 Cepheid Headquarters Locations

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22 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

Australia

Email: techsupportANZ@cepheid.com

Tech Support: TOLLFREE (Aust)

1800 130 821

Fax: 1300 798 459

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en_US/support/contact-us.

23 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
°C	Temperature limitation
	Biological risks

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IVD

24 Revision History

Description of Changes: 302-8927, Rev. A

Purpose: Initial release of Instructions for Use

GeneXpert
Powered By CEPHEID INNOVATION

Xpert® Xpress SARS-CoV-2/ Flu/RSV

REF **XPCOV2/FLU/RSV-10**

Instructions for Use

For Use with GeneXpert® Dx or GeneXpert® Infinity Systems

IVD CE

Cepheid

For *In Vitro* Diagnostic Use

302-5159, Rev. D
May 2022

Trademark, Patents, and Copyright Statements

See Section 26, Revision History for a description of changes.

Xpert® Xpress SARS-CoV-2/Flu/RSV

1 Proprietary Name

Xpert® Xpress SARS-CoV-2/Flu/RSV

2 Common or Usual Name

Xpert Xpress SARS-CoV-2/Flu/RSV

3 Intended Use

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a multiplexed real-time RT-PCR test intended for the simultaneous, qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA in either nasopharyngeal swab, nasal swab or nasal wash/aspirate specimens collected from individuals suspected of respiratory viral infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus and RSV RNA in clinical specimens. Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and/or epidemiological information.

4 Summary and Explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.[1] Chinese authorities identified a novel coronavirus (2019-nCoV), which has since spread globally, resulting in a pandemic of coronavirus disease 2019 (COVID-19). COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection, mild upper respiratory infection, severe lower respiratory disease including pneumonia and respiratory failure, and in some cases, death. The International Committee on Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.[2]

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[3,4]

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.[5] Flu A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by influenza A subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the Pneumoviridae family (formerly Paramyxoviridae), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, and the elderly who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).[6] The virus can remain infectious for hours on countertops and toys and can cause both

upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia.[6] By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected.[6] Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season mirrors influenza somewhat as infections begin to rise during the fall through early spring.[5,6]

Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza and RSV. The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a molecular in vitro diagnostic test that aids in the detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2/Flu/RSV test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus in upper respiratory specimens.

5 Principle of the Procedure

The Xpert Xpress SARS-CoV-2/Flu/RSV test is an automated in vitro diagnostic test for qualitative detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus. The Xpert Xpress SARS-CoV-2/Flu/RSV test is performed on GeneXpert Instrument Systems. The primers and probes in the Xpert Xpress SARS-CoV-2/Flu/RSV test are designed to amplify and detect unique sequences in the following: nucleocapsid (N2) and envelope (E) genes of the SARS-CoV-2 virus genome, influenza A matrix (M), influenza A basic polymerase (PB2), influenza A acidic protein (PA), influenza B matrix (M), influenza B non-structural protein (NS), and the RSV A and RSV B nucleocapsid.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR and RT-PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The Xpert Xpress SARS-CoV-2/Flu/RSV test includes reagents for the detection of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus in either nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal swab, nasal swab, or nasal wash/ aspirate specimen is collected and placed into a transport tube containing 3 mL of viral transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

6 Reagents and Instruments

6.1 Materials Provided

The Xpert Xpress SARS-CoV-2/Flu/RSV kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

Xpert Xpress SARS-CoV-2/Flu/RSV Cartridges with Integrated Reaction Tubes	**10**
Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
Lysis Reagent	1.0 mL per cartridge
Binding Reagent	1.0 mL per cartridge
Elution Reagent	3.0 mL per cartridge
Wash Reagent	0.4 mL per cartridge
Disposable Transfer Pipettes	**10-12 per kit**
Flyer	**1 per kit**
Instructions to locate (and import) the ADF and documentation such as the product insert on www.cepheid.com.	
Quick Reference Instructions	**2 per kit**
For use with the GeneXpert Xpress System only	

Note Safety Data Sheets (SDS) are available at www.cepheid.com or www.cepheidinternational.com under the **SUPPORT** tab.

Note The bovine serum albumin (BSA) in the beads within this product was produced and manufactured exclusively from bovine plasma sourced in the United States. No ruminant protein or other animal protein was fed to the animals; the animals passed ante- and post-mortem testing. During processing, there was no mixing of the material with other animal materials.

7 Storage and Handling

- Store the Xpert Xpress SARS-CoV-2/Flu/RSV cartridges at 2-28 °C.
- Do not open a cartridge lid until you are ready to perform testing.
- Do not use a cartridge that is wet or has leaked.

8 Materials Required but Not Provided

- Nylon flocked swab (Copan P/N 502CS01, 503CS01) or equivalent
- Viral transport medium, 3 mL (Copan P/N 330C) or equivalent
- 0.9% (w/v) saline, 3 mL
- Nasopharyngeal Sample Collection Kit for Viruses (Cepheid P/N SWAB/B-100, Copan P/N 305C) or equivalent
- Nasal Sample Collection Kit for Viruses (Cepheid P/N SWAB/F-100, Copan P/N 346C) or equivalent
- GeneXpert Dx or GeneXpert Infinity systems (catalog number varies by configuration): GeneXpert instrument, computer, barcode scanner, operator manual.

  For GeneXpert Dx System: GeneXpert Dx software version 4.7b or higher

  For GeneXpert Infinity-80 and Infinity-48s systems: Xpertise software version 6.4b or higher

9 Materials Available but Not Provided

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY).

- External Positive Control: Catalog #NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)
- External Negative Control: Catalog #NATCV9-6C (Coxsackievirus A9)

10 Warnings and Precautions

10.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of presence of Flu A, Flu B, RSV or SARS-CoV-2-RNA.
- Performance characteristics of this test have been established with nasopharyngeal and nasal swab specimen types. The performance of this assay with other specimen types or samples has not been evaluated.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention[7] and the Clinical and Laboratory Standards Institute.[8]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. Institutions should check the hazardous waste disposal requirements within their respective countries.

10.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 12. Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

10.3 Assay/Reagent

- Do not open the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping the cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the Sample ID label on the cartridge lid or on the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Do not use reagents beyond their expiry date.
- Each single-use Xpert Xpress SARS-CoV-2/Flu/RSV cartridge is used to process one test. Do not reuse processed cartridges.
- Each single-use disposable pipette is used to transfer one specimen. Do not reuse disposable pipettes.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- In the event of a spill of specimens or controls, wear gloves and absorb the spill with paper towels. Then, thoroughly clean the contaminated area with a 10% freshly prepared household chlorine bleach. Allow a minimum of two minutes of contact time. Ensure the work area is dry before using 70% denatured ethanol to remove bleach residue. Allow surface to dry completely before proceeding. Or, follow your institution’s standard procedures for a contamination or spill event. For equipment, follow the manufacturer’s recommendations for decontamination of equipment.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution’s environmental waste procedures for proper disposal of used cartridges and unused reagents. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

11 Chemical Hazards[9,10]

Signal Word: WARNING

UN GHS Hazard Statements

- Harmful if swallowed.
- May be harmful in contact with skin.
- Causes eye irritation.

UN GHS Precautionary Statements

Prevention

- Wash hands thoroughly after handling.

Response

- Call a POISON CENTER or doctor/physician if you feel unwell.
- If skin irritation occurs: Get medical advice/attention.
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If eye irritation persists: Get medical advice/attention.

12 Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 12.1 for nasopharyngeal swab collection procedure, Section 12.2 for nasal swab collection procedure, and Section 12.3 for nasal wash/aspirate procedure.

Nasopharyngeal swab, nasal swab, and nasal wash/aspirate specimens can be stored at room temperature (15-30 °C) for up to 24 hours in viral transport medium or 48 hours in saline until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal swab, nasal swab, and nasal wash/aspirate specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium or saline until testing is performed on the GeneXpert Instrument Systems.

Refer to the WHO Laboratory Biosafety Guidance Related to the Coronavirus Disease 2019 (COVID-19). https://www.who.int/publications-detail/laboratory-biosafety-guidance-realted-to-coronavirus-disease-2019-(covid-19)

12.1 Nasopharyngeal Swab Collection Procedure

1. Insert the swab into either nostril, passing it into the posterior nasopharynx (see Figure 1).

Figure 1. Nasopharyngeal Swab Collection

2. Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline.
3. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.2 Nasal Swab Collection Procedure

1. Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril (see Figure 2).

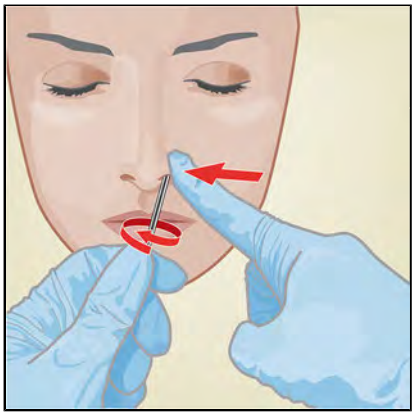

Figure 2. Nasal Swab Collection for First Nostril

2. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril (see Figure 3). To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.

Figure 3. Nasal Swab Collection for Second Nostril

3. Remove and place the swab into the tube containing 3 mL of viral transport medium or 3 mL of saline. Break swab at the indicated break line and cap the specimen collection tube tightly.

12.3 Nasal Wash/Aspirate Procedure

1. Nasal wash/aspirate specimens can be collected following the user institution's standard procedure. Also, refer to the WHO guidelines for the collection of human nasal wash/aspirate specimens.
2. https://www.who.int/influenza/human_animal_interface/virology_laboratories_and_vaccines/guidelines_collection_h5n1_humans/en/
3. Using a clean transfer pipette, transfer 600 µL of the sample into the tube containing 3 mL of viral transport medium or 3 mL of saline and then cap the tube.

13 Procedure

13.1 Preparing the Cartridge

Important **Start the test within 30 minutes of adding the sample to the cartridge.**

1. Remove a cartridge from the package.

2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open the cap on the specimen transport tube.
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette **completely until the top bulb is fully flat**. While continuing to hold the bulb fully flat, place the pipette tip in the specimen transport tube (see Figure 4).

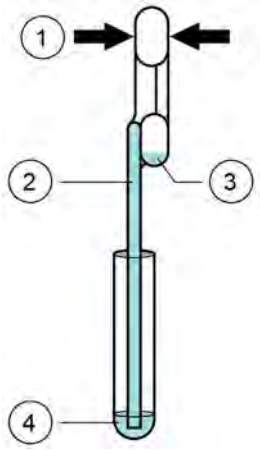

Figure 4. Transfer Pipette

Number	Description
1	Squeeze here.
2	Pipette
3	Overflow Reservoir Bulb
4	Sample

7. Keeping the pipette below the surface of the liquid, release the top bulb of the pipette slowly to fill the pipette with sample before removing from the tube. It is okay if liquid goes into the overflow reservoir (see Figure 4). Check that the pipette does not contain bubbles.
8. To transfer the sample to the cartridge, squeeze the top bulb of the pipette completely again until it is fully flat to empty the contents of the pipette (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5. Some liquid may remain in the overflow reservoir. Dispose of the used pipette.

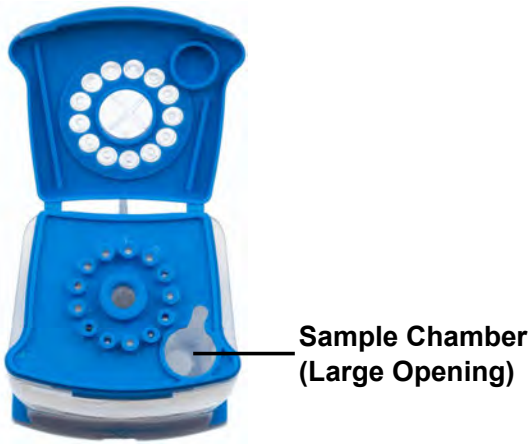

Figure 5. Xpert Xpress SARS-CoV-2/Flu/RSV Cartridge (Top View)

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

9. Close the cartridge lid.

13.2 External Controls

External controls described in Section 9 are available, but not provided and may be used in accordance with local, state, and federal accrediting organizations, as applicable.

To run a control using the Xpert Xpress SARS-CoV-2/Flu/RSV test, perform the following steps:

1. Mix control by rapidly inverting the external control tube 5 times. Open cap on external control tube.
2. Open the cartridge lid.
3. Using a clean transfer pipette, transfer one draw of the external control sample (300 µL) into the large opening (Sample Chamber) in the cartridge shown in Figure 5.
4. Close cartridge lid.

13.3 Starting the Test

Note Before you start the test, make sure that the system contains modules with GeneXpert Dx software version 4.7b or higher or Infinity Xpertise software 6.4b or higher, and that the Xpert Xpress SARS-CoV-2/Flu/RSV Assay Definition File is imported into the software.

Note This section lists the default steps to operate the GeneXpert Instrument System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

Note The steps you follow may be different if the system administrator has changed the default workflow of the system.

1. Turn on the GeneXpert Instrument System:
   - If using the *GeneXpert Dx instrument*, first turn on the instrument, and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double-clicking on the GeneXpert Dx shortcut icon on the Windows® desktop.

     or
   - If using the *GeneXpert Infinity instrument*, power up the instrument by turning the power switch clockwise to the ON position. On the Windows desktop, double click the Xpertise software shortcut icon to launch the software.
2. Log on to the System software. The login screen appears. Type your user name and password.
3. In the **GeneXpert System** window, click **Create Test** (GeneXpert Dx) or **Orders** followed by **Order Test** (Infinity).
4. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown in the **View Results** window and is associated with the test reports.
5. Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is shown on the left side of the View Results window and is associated with the test result.
6. Scan the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date, and Selected Assay.

Note If the barcode on the Xpert Xpress SARS-CoV-2/Flu/RSV cartridge does not scan, then repeat the test with a new cartridge.

7. Click **Start Test** (GeneXpert Dx) or Submit (Infinity) if Auto-Submit is not enabled. In the dialog box that appears, type your password, if required.

   For the *GeneXpert Dx Instrument*:

   a) Locate the module with the blinking green light, open the instrument module door and load the cartridge.
   b) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

c) Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.

For the GeneXpert Infinity System

a) After clicking **Submit**, you will be asked to place the cartridge on the conveyor belt. After placing the cartridge, click **OK** to continue. The cartridge will be automatically loaded, the test will run and the used cartridge will be placed onto the waste shelf for disposal.

b) When all samples are loaded, click on the **End Order Test** icon.

Note Do not turn off or unplug the instruments while a test is in progress. Turning off or unplugging the GeneXpert instrument or computer will stop the test.

14 Viewing and Printing Results

For detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

15 Quality Control

15.1 Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

15.2 External Controls

External controls should be used in accordance with local, state, and federal accrediting organizations as applicable.

16 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2/Flu/RSV test provides test results based on the detection of respective gene targets according to the algorithms.

The format of the test results presented will vary depending on the user's choice to run either an Xpert Xpress_SARS-CoV-2_Flu_RSV, Xpert Xpress_SARS-CoV-2_Flu or Xpert Xpress_SARS-CoV-2 test.

Table 1 shows the possible result outcomes when the **Xpert Xpress SARS-CoV-2_Flu_RSV** test mode is selected.

Table 1. Xpert Xpress SARS-CoV-2_Flu_RSV Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. ● SPC - NA; SPC is ignored because the Flu A target amplification occurred. ● Probe Check - PASS; all probe check results pass.
Flu B POSITIVE	● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred. ● Probe Check: PASS; all probe check results pass.
RSV POSITIVE	● The RSV signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because RSV target amplification occurred. ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. ● SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. ● SPC - PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 17.2. ● SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signals do not have a Ct within valid range and endpoint is below minimum setting. ● Probe Check - PASS; all probe check results pass.

Result	Interpretation
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[a]; all or one of the probe check results fail.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● RSV: NO RESULT ● SPC: NO RESULT ● Probe Check: NA
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

[a] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Table 2 shows the possible result outcomes when the **Xpert Xpress_SARS-CoV-2_Flu** test mode is selected.

Table 2. Xpert Xpress_SARS-CoV-2_Flu Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. ● Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	● The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. ● SPC - NA; SPC is ignored because the Flu A target amplification occurred. ● Probe Check - PASS; all probe check results pass.
Flu B POSITIVE	● The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA; SPC is ignored because Flu B target amplification occurred. ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected. ● SARS-CoV-2, Flu A, and Flu B target RNAs are not detected. ● SPC - PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 17.2. ● SPC: FAIL; SPC and SARS-CoV-2, Flu A, and Flu B signals do not have a Ct within valid range and endpoint is below minimum setting. ● Probe Check - PASS; all probe check results pass.
ERROR	Presence or absence of SARS-CoV-2, Flu A, and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: FAIL[a]; all or one of the probe check results fail.

Result	Interpretation
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● Flu A: NO RESULT ● Flu B: NO RESULT ● SPC: NO RESULT ● Probe Check: NA
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

[a] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

Table 3 shows the possible result outcomes when the **Xpert Xpress_SARS-CoV-2** test mode is selected.

Table 3. Xpert Xpress_SARS-CoV-2 Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. ● The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. ● SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. ● Probe Check: PASS; all probe check results pass.
SARS-CoV-2 NEGATIVE	SARS-CoV-2 target RNA is not detected. ● SARS-CoV-2 target RNA is not detected. ● SPC – PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. ● Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and SARS-CoV-2 is not detected. Repeat test according to the Retest Procedure in Section 17.2. ● SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint is below minimum setting. ● Probe Check - PASS; all probe check results pass.
ERROR	Presence or absence of SARS-CoV-2 RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. ● SPC: NO RESULT ● Probe Check: FAIL[a]; all or one of the probe check results fail.

Result	Interpretation
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, and Flu B RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. ● SARS-CoV-2: NO RESULT ● SPC: NO RESULT ● Probe Check: NA

[a] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.

The Xpert Xpress SARS-CoV-2/Flu/RSV test can be run to detect SARS-CoV-2, Flu and RSV by selecting **Xpert Xpress_SARS-CoV-2_Flu_RSV** from the **Select Test** menu; SARS-CoV-2 and Flu only by selecting **Xpert Xpress_SARS-CoV- 2_Flu**; or SARS-CoV-2 only by selecting **Xpert Xpress_SARS-CoV-2**. The Xpert Xpress_SARS-CoV-2 test mode includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens if the signal from the SARS-CoV-2 target reaches a predetermined threshold before the full 45 PCR cycles have been completed. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

17 Retests

17.1 Reasons to Repeat the Test

If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

- An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
- An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, no sample added, or the maximum pressure limits were exceeded.
- A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

17.2 Retest Procedure

To retest a non-determinate result (**INVALID**, **NO RESULT**, or **ERROR**), use a new cartridge.

Use the leftover sample from the original specimen transport medium tube or new external control tube.

1. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2/Flu/RSV cartridge and a new transfer pipette.
2. Check the specimen transport tube or external control tube is closed.
3. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
4. Open the cartridge lid.
5. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
6. Close the cartridge lid.

18 Limitations

- Performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test has only been established in nasopharyngeal and nasal swab specimens. Use of the Xpert Xpress SARS-CoV-2/Flu/RSV test with other specimen types has not been assessed and performance characteristics are unknown.
- Nasal wash/aspirate specimens are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2/Flu/RSV test but performance with these specimen types has not been established.
- As with any molecular test, mutations within the target regions of the Xpert Xpress SARS-CoV-2/Flu/RSV test could affect primer and/or probe binding resulting in failure to detect the presence of virus or the virus being detected less predictably.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
- False negative results may occur if virus is present at levels below the analytical limit of detection.
- Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
- Results from the Xpert Xpress SARS-CoV-2/Flu/RSV test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- Viral nucleic acid may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
- This test has been evaluated for use with human specimen material only.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- This test has not been evaluated for monitoring treatment of infection.
- This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza or RSV.
- The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
- Results from analytical studies with contrived co-infected samples showed potential for competitive interference when SARS-CoV-2, influenza or RSV was present at 1X LoD levels.
- Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
- As the Xpert Xpress SARS-CoV-2/Flu/RSV test does not differentiate between the N2 and E gene targets, the presence of other coronaviruses in the B lineage, Betacoronavirus genus, including SARS-CoV-1 may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
- This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Specimen transport media that contain guanidine thiocyanate (GTC) may interfere with the test causing false negative results.
- Alternate transport media, not identified in this package insert, should be validated for use with Xpert Xpress SARS-CoV-2/Flu/RSV.
- Any alternate transport media that have been previously validated with other Cepheid tests may still require validation for use with Xpert Xpress SARS-CoV-2/Flu/RSV.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.

19 Performance Characteristics

19.1 Clinical Evaluation

The performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test was evaluated using archived clinical nasopharyngeal (NP) swab and nasal swab (NS) specimens in viral transport medium. Archived specimens were selected consecutively by date and previously known analyte result. A total of 240 NP swab specimens and 239 NS specimens were tested with Xpert Xpress SARS-CoV-2/Flu/RSV side by side with a CE-marked SARS-CoV-2 RT-PCR test and the Xpert Xpress Flu/RSV test in a randomized and blinded fashion.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the Xpert Xpress SARS-CoV-2/Flu/RSV test relative to the results of a CE-marked SARS-CoV-2 RT-PCR test for the SARS-CoV-2 target, and Xpert Xpress Flu/RSV for the Flu A, Flu B, and RSV targets, respectively.

For the NP swab specimens,Xpert Xpress SARS-CoV-2/Flu/RSV demonstrated a PPA and NPA of 97.9% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 99.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 4).

Table 4. Xpert Xpress SARS-CoV-2/Flu/RSV Performance Results Using NP Swab Specimens

Target	Number of NP Swab Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	240	46	0	193	1	97.9% (88.9% - 99.6%)	100.0% (98.1% - 100.0%)
Flu A	240	48	0	192	0	100% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
Flu B	240	46	2	192	0	100.0% (92.3% - 100.0%)	99.0% (96.3% - 99.7%)
RSV	240	47	0	193	0	100.0% (92.4% - 100.0%)	100.0% (98.1% - 100.0%)

TP: True Positive; FP: False Positive; TN: True Negative; FN: False Negative; CI: Confidence Interval

For the NS specimens, Xpert Xpress SARS-CoV-2/Flu/RSV demonstrated a PPA and NPA of 97.9% and 100.0% for SARS-CoV-2, respectively; 100.0% and 100.0% for Flu A, respectively; 100.0% and 100.0% for Flu B, respectively; 100.0% and 100.0% for RSV, respectively (Table 5).

Table 5. Xpert Xpress SARS-CoV-2/Flu/RSV Performance Results Using NS Swab Specimens

Target	Number of NS Swab Specimens	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	239	47	0	191	1	97.9% (89.1% - 99.6%)	100.0% (98.0% - 100.0%)
Flu A	239	48	0	191	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)
Flu B	239	47	0	192	0	100.0% (92.4% - 100.0%)	100.0% (98.0% - 100.0%)
RSV	239	48	0	191	0	100.0% (92.6% - 100.0%)	100.0% (98.0% - 100.0%)

20 Analytical Performance

20.1 Analytical Sensitivity (Limit of Detection)

The analytical sensitivity of the Xpert Xpress SARS-CoV-2/Flu/RSV test was assessed with one lot of reagent and limiting dilutions of the six respiratory viruses (NATtrol SARS-CoV-2, Flu A H1, Flu A H3, Flu B, RSV A and RSV B) into pooled negative clinical NP swab matrix following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. The estimated LoD values as determined by Probit regression analysis were verified using two lots of Xpert Xpress SARS-CoV-2/Flu/RSV reagents. The verified LoD values for the viruses tested are summarized in Table 6.

Table 6. Xpert Xpress SARS-CoV-2/Flu/RSV Limit of Detection

Virus/Strain	LoD Concentration
SARS-CoV-2 (USA-WA1/2020)	131 copies/mL
Influenza A/California/7/2009	0.004 $TCID_{50}$/mL
Influenza A/Victoria/361/2011	0.087 $TCID_{50}$/mL
Influenza B/Mass/2/2012	0.04 $TCID_{50}$/mL
RSV A/2/Australia/61	0.43 $TCID_{50}$/mL
RSV B/Wash/18537/62	0.22 $TCID_{50}$/mL

20.2 Analytical Reactivity (Inclusivity)

The inclusivity of Xpert Xpress SARS-CoV-2/Flu/RSV was evaluated using *in silico* analysis of the assay amplicons in relation to 48,461 SARS-CoV-2 sequences available in the GISAID gene database for two targets, E and N2.

For analysis of the E target, 113 sequences were excluded due to ambiguous nucleotides, which reduced the total to 48,348 sequences. Of the 48,348 GISAID sequences, 48,108 (99.5%) were an exact match to the SARS-CoV-2 E target amplicon generated in the Xpert Xpress SARS-CoV-2/Flu/RSV test. Single nucleotide mismatches were observed for 223 sequences and two mismatches were observed for 17 sequences. Of the 17 sequences with two mismatches, two sequences contained 2 mismatches in the forward primer region, three sequences have a ‘GA' dinucleotide in the reverse primer, and twelve sequences contained a ‘AA’ dinucleotide that lies between the oligonucleotides used in the assay. None of these mismatches are expected to affect the performance of the assay.

For analysis of the N2 target, 129 sequences were excluded due to ambiguous nucleotides, which reduced the total used in the evaluation to 48,332 sequences. Of the 48,332 GISAID sequences, 47,962 (99.2%) were an exact match to the SARS-CoV-2 N2 target amplicon generated in the Xpert Xpress SARS-CoV-2/Flu/RSV test. Single nucleotide mismatches were observed for 369 sequences and three (3) mismatches were observed for one sequence. For the one sequence with three variant positions, two of the mismatched nucleotides are in the probe region and could have an impact on probe binding. None of the other mismatches are predicted to have a negative impact on the performance of the assay.

The inclusivity of the Xpert Xpress SARS-CoV-2/Flu/RSV for Flu and RSV viruses are as reported for the analytical reactivity evaluation of the Xpert Xpress Flu/RSV test.

Xpert Xpress Flu/RSV test was evaluated against multiple strains of influenza A H1N1 (seasonal pre-2009), influenza A H1N1 (pandemic 2009), influenza A H3N2 (seasonal), avian influenza A (H5N1, H5N2, H6N2, H7N2, H7N3, H2N2, H7N9, and H9N2), influenza B (representing strains from both Victoria and Yamagata lineages), and respiratory syncytial virus subgroups A and B (RSV A and RSV B) at levels near the analytical LoD. A total of 53 strains comprised of 48 influenza viruses (35 influenza A and 13 influenza B) and 5 RSV strains were tested in this study with the Xpert Xpress Flu/RSV test. Three replicates were tested for each strain. All Flu and RSV strains tested positive in all three replicates, except for one Flu A H1N1 strain (A/New Jersey/8/76), which tested positive in 2 of 3 replicates at 0.1 $TCID_{50}$/mL. Results are shown in Table 7. Predicted cross reactivity from *in silico* analyses showed 100% sequence homology for additional pH1N1 strains.

Table 7. Analytical Reactivity (Inclusivity) of the Xpert Xpress Flu/RSV Test

Virus	Strain	Target Concentration	Result		
			Flu A	Flu B	RSV
No Template Control		N/A	NEG	NEG	NEG
Influenza A H1N1 (pre-2009)	A/swine/Iowa/15/30	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/WS/33	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/PR/8/34	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Mal/302/54	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Denver/1/57	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Jersey/8/76	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New Caledonia/20/1999	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/New York/55/2004	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Solomon Island/3/2006	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Taiwan/42/06	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/59/2007	0.1 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H1N1 (pdm2009)	A/swine/NY/02/2009	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Colorado/14/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG
	A/Washington/24/2012	0.1 $TCID_{50}$/mL	POS	NEG	NEG
Influenza A H3N2 (Seasonal)	A/Aichi/2/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hong Kong/8/68	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Port Chalmers/1/73	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Hawaii/15/2001	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Wisconsin/67/05	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Brisbane/10/2007	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Minnesota/11/2010 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Indiana/08/2011 (H3N2)v	2.0 $TCID_{50}$/mL	POS	NEG	NEG
	A/Texas/50/2012	2.0 $TCID_{50}$/mL	POS	NEG	NEG
Avian influenza A	A/duck/Hunan/795/2002 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/Hubei/327/2004 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Anhui/01/2005 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Japanese white eye/Hong Kong/1038/2006 (H5N1)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/mallard/WI/34/75 (H5N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/CA431/00 (H6N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/duck/LTC-10-82743/1943 (H7N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/chicken/NJ/15086-3/94 (H7N3)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Anhui/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG

Virus	Strain	Target Concentration	Result		
			Flu A	Flu B	RSV
	A/Shanghai/1/2013 (H7N9)	N/A[b]	POS	NEG	NEG
	A/chicken/Korea/38349-p96323/1996(H9N2)	≤ 1pg/µL[a]	POS	NEG	NEG
	A/Mallard/NY/6750/78 (H2N2)	≤ 1pg/µL[a]	POS	NEG	NEG
Influenza B	B/Lee/40	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Allen/45	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/GL/1739/54	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Maryland/1/59	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Panama/45/90[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/07/2004[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/02/06[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Florida/04/06[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Hong Kong/5/72	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Wisconsin/01/2011[d]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Malaysia/2506/04[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Taiwan/2/62	1.0 $TCID_{50}$/mL	NEG	POS	NEG
	B/Brisbane/60/2008[c]	1.0 $TCID_{50}$/mL	NEG	POS	NEG
RSV A	RSV-A/NY (Clinical unknown)	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-8-2/2007	3.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-A/WI/629-11-1/2008	3.0 $TCID_{50}$/mL	NEG	NEG	POS
RSV B	RSV-B/WV14617/85	7.0 $TCID_{50}$/mL	NEG	NEG	POS
	RSV-B/CH93(18)-18	7.0 $TCID_{50}$/mL	NEG	NEG	POS

[a] Purified viral RNA in simulated background matrix was used for avian influenza A viruses due to biosafety regulations.
[b] Inactivated avian influenza A (H7N9) viruses without viral titer was diluted 100,000-fold in simulated background matrix and tested due to biosafety regulations.
[c] Known Victoria lineage.
[d] Known Yamagata lineage.

20.3 Analytical Specificity (Exclusivity)

An *in silico* analysis for possible cross-reactions with all the organisms listed in Table 8 was conducted by mapping primers and probes in the Xpert Xpress SARS-CoV-2/Flu/RSV test individually to the sequences downloaded from the GISAID database. E primers and probes are not specific for SARS-CoV-2 and will detect Human and Bat SARS-coronavirus. No potential unintended cross reactivity with other organisms listed in Table 8 is expected based on the *in silico* analysis.

Table 8. Xpert Xpress SARS-CoV-2/Flu/RSV Analytical Specificity Microorganisms

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza viruses 1-4

Microorganisms from the Same Genetic Family	High Priority Organisms
Human coronavirus NL63	Influenza A
SARS-coronavirus	Influenza B
MERS-coronavirus	Influenza C
Bat coronavirus	Enterovirus (e.g. EV68)
	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Parechovirus
	Candida albicans
	Corynebacterium diphtheriae
	Legionella non-*pneumophila*
	Bacillus anthracis (Anthrax)
	Moraxella catarrhalis
	Neisseria elongata and N. meningitidis
	Pseudomonas aeruginosa
	Staphylococcus epidermidis
	Streptococcus salivarius
	Leptospira
	Chlamydia psittaci
	Coxiella burnetii (Q-Fever)
	Staphylococcus aureus

The analytical specificity of the Xpert Xpress SARS-CoV-2/Flu/RSV for Flu A, Flu B and RSV viruses are as reported for the analytical exclusivity evaluation of the Xpert Xpress Flu/RSV test. The analytical specificity of the Xpert Xpress Flu/RSV test was evaluated by testing a panel of 44 cultures consisting of 16 viral, 26 bacterial, and two yeast strains representing common respiratory pathogens or those potentially encountered in the nasopharynx. Three replicates of each bacterial and yeast strain were tested at concentrations of $\geq 1 \times 10^6$ CFU/mL with the exception of one strain that was tested at 1×10^5 CFU/mL (*Chlamydia pneumoniae*). Three replicates of each virus were tested at concentrations of $\geq 1 \times 10^5$ TCID 50 /mL. The analytical specificity was 100%. Results are shown in Table 9.

Table 9. Analytical Specificity of the Xpert Xpress Flu/RSV Test

Organism	Concentration	Influenza A	Influenza B	RSV
No Template Control	N/A	NEG	NEG	NEG
Adenovirus Type 1	1.12E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Adenovirus Type 7	1.87E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus OC43	2.85E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human coronavirus 229E	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Cytomegalovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Echovirus	3.31E+07 $TCID_{50}$/mL	NEG	NEG	NEG
Enterovirus	3.55E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Epstein Barr Virus	7.16E+07 $TCID_{50}$/mL	NEG	NEG	NEG
Herpes simplex virus	8.90E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Measles	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human metapneumovirus	1.00E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Mumps virus	6.31E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza virus Type 1	1.15E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza virus Type 2	6.31E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Human parainfluenza virus Type 3	3.55E+06 $TCID_{50}$/mL	NEG	NEG	NEG
Rhinovirus Type 1A	1.26E+05 $TCID_{50}$/mL	NEG	NEG	NEG
Acinetobacter baumannii	1.00E+06 CFU/mL	NEG	NEG	NEG
Burkholderia cepacia	3.30E+06 CFU/mL	NEG	NEG	NEG
Candida albicans	3.20E+06 CFU/mL	NEG	NEG	NEG
Candida parapsilosis	3.00E+06 CFU/mL	NEG	NEG	NEG
Bordetella pertussis	3.30E+06 CFU/mL	NEG	NEG	NEG
Chlamydia pneumoniae	1.00E+05 CFU/mL	NEG	NEG	NEG
Citrobacter freundii	3.30E+06 CFU/mL	NEG	NEG	NEG
Corynebacterium sp.	3.30E+06 CFU/mL	NEG	NEG	NEG

Organism	Concentration	Influenza A	Influenza B	RSV
Escherichia coli	1.00E+07 CFU/mL	NEG	NEG	NEG
Enterococcus faecalis	1.30E+06 CFU/mL	NEG	NEG	NEG
Hemophilus influenzae	1.00E+06 CFU/mL	NEG	NEG	NEG
Lactobacillus reuteri	1.00E+06 CFU/mL	NEG	NEG	NEG
Legionella spp.	1.00E+06 CFU/mL	NEG	NEG	NEG
Moraxella catarrhalis	1.00E+07 CFU/mL	NEG	NEG	NEG
Mycobacterium tuberculosis (avirulent)	1.00E+06 CFU/mL	NEG	NEG	NEG
Mycoplasma pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Neisseria meningitidis	2.15E+06 CFU/mL	NEG	NEG	NEG
Neisseria mucosa	1.00E+07 CFU/mL	NEG	NEG	NEG
Propionibacterium acnes	2.40E+07 CFU/mL	NEG	NEG	NEG
Pseudomonas aeruginosa	3.70E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus aureus (protein A producer)	2.20E+06 CFU/mL	NEG	NEG	NEG
Staphylococcus epidermidis	3.40E+06 CFU/mL	NEG	NEG	NEG
Staphyolcoccus haemolyticus	4.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus agalactiae	3.50E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pneumoniae	1.00E+06 CFU/mL	NEG	NEG	NEG
Streptococcus pyogenes	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus salivarius	1.00E+07 CFU/mL	NEG	NEG	NEG
Streptococcus sanguinis	3.10E+06 CFU/mL	NEG	NEG	NEG

20.4 Competitive Interference

Competitive interference of the Xpert Xpress SARS-CoV-2/Flu/RSV caused by co-infections were evaluated by testing individual SARS-CoV-2, Flu A, Flu B or RSV strains at 1X LoD in the presence of different target strains at a higher concentration in a simulated background matrix. The concentration at LoD was 131 copies/mL for SARS-CoV-2 and ranged from 0.004 $TCID_{50}$/mL to 0.43 $TCID_{50}$/mL for Flu and RSV strains; the competitive strains were evaluated at 10^4 titer units (copies/mL, $TCID_{50}$/mL, $CEID_{50}$/mL or PFU/mL). The corresponding concentration of RNA (copies/mL) for the Flu and RSV strains was determined by ddPCR.

Analytical competitive interference was assessed using a strain of SARS-CoV-2 (inactivated USA-WA1/2020), Flu A H3 (H3/Victoria/361/2011), Flu B (B/Mass/02/2012), RSV A (RSV-A/2/Australia/ 61), and RSV B (RSV-B/Wash/18537/62). Replicates of 20 were tested for each target strain and each competitive strain combination. The normal binomial distribution with 20 replicate samples at LoD is between 17 and 20 positive results based on the binomial distribution with N=20, p=0.95 (X~Bin(20,0.95)). Therefore, sets of 20 with 16 or less positives would be rare and an indication of a competitive inhibitory effect due to high levels of a competing analyte. Below is a summary of the results:

Table 10. Summary of Results for Competitive Interference

		Correct Calls (n/20)					
		Test Strain at LoD and Interferent at:					
Test Strain at LoD	**Interferent Strain**	**10^4 * (2.1e7 cp/mL)**	**10^3 * (2.1e6 cp/mL)**	**10^2 * (2.1e5 cp/mL)**	**10 * (2.1e4 cp/mL)**	**1 * (2.1e3 cp/mL)**	**0.1 * (2.1e2 cp/mL)**
Flu B	Flu A	*6/20*	**20/20**				
RSV A	Flu A	*9/20*	**17/20**				
RSV B	Flu A	*11/20*	**18/20**				
SARS-CoV-2	Flu A	*6/20*	**17/20**	**20/20**			
Test Strain at LoD	**Interferent Strain**	**10^4 * (5.2e7 cp/mL)**	**10^3 * (5.2e6 cp/mL)**	**10^2 * (5.2e5 cp/mL)**	**10 * (5.2e4 cp/mL)**	**1 * (5.2e3 cp/mL)**	**0.1 * (5.2e2 cp/mL)**
Flu A	Flu B	*1/20*	*4/20*	*8/20*	*9/19*	*15/20*	**20/20**
RSV A	Flu B	*0/20*	*0/20*	*3/20*	**18/20**		
RSV B	Flu B	*7/20*	*8/20*	*11/20*	**18/20**		
SARS-CoV-2	Flu B	*3/20*	*4/20*	*11/20*	**17/20**	**20/20**	
Test Strain at LoD	**Interferent Strain**	**10^4 * (3.7e7 cp/mL)**	**10^3 * (3.7e6 cp/mL)**	**10^2 * (3.7e5 cp/mL)**	**10 * (3.7e4 cp/mL)**	**1 * (3.7e3 cp/mL)**	**0.1 * (3.7e2 cp/mL)**
Flu A	RSV A	*15/20*	**12/20**	**20/20**			
Flu B	RSV A	*15/20*	**17/20**				
SARS-CoV-2	RSV A	**17/20**	**19/20**				
Test Strain at LoD	**Interferent Strain**	**10^4 * (1.1e7 cp/mL)**	**10^3 * (1.1e6 cp/mL)**	**10^2 * (1.1e5 cp/mL)**	**10 * (1.1e4 cp/mL)**	**1 * (1.1e3 cp/mL)**	**0.1 * (1.1e2 cp/mL)**
Flu A	RSV B	*9/20*	*7/20*	*6/20*	*14/20*	**20/20**	
Flu B	RSV B	*10/20*	*10/20*	*16/20*	**19/20**		
SARS-CoV-2	RSV B	**17/20**	*16/20*	*15/20*	**20/20**		
Test Strain at LoD	**Interferent Strain**	**10^4 ***	**10^3 ***	**10^2 ***	**10 ***	**1 ***	**0.1 ***
Flu A	SARS-CoV-2	**19/20**					
Flu B	SARS-CoV-2	**18/20**					
RSV A	SARS-CoV-2	**19/20**					
RSV B	SARS-CoV-2	**19/20**					

* Units for the concentration of each organism are as follows: Flu A H3 - $CEID_{50}$/mL; Flu B and RSV B - $TCID_{50}$/mL; RSV A- PFU/mL; SARS-CoV-2 - copies/mL

Italicized font indicates inhibitory effects

Bold font indicates no inhibition (SARS-CoV-2 tested to >19/20)

Flu A/Victoria/361/2011 at a concentration of 1 x 10^4 $CEID_{50}$/mL (2.1e7 copies/mL), inhibited Flu B, RSV A, RSV B and SARS-CoV-2 at the LoD.

Flu B/Mass/2/2012 at concentrations shown in Table 10, inhibited SARS-CoV-2, Flu A, RSV A and RSV B at concentrations at the LoD of those targets.

RSV A/2/Australia/61 at a concentration of 1 x 10^4 PFU/mL (3.7e7 copies/mL), inhibited SARS-CoV-2, Flu A and Flu B at the LoD.

RSV-B/Wash/18537/62 at concentrations shown in Table 10, inhibited SARS-CoV-2, Flu A and Flu B at concentrations at the LoD of those targets.

20.5 Potentially Interfering Substances

Potentially interfering substances that could be present in the nasopharynx (or introduced during specimen collection and handling) and interfere with accurate detection of SARS-CoV-2, Flu A, Flu B and RSV were evaluated with select direct testing on the Xpert Xpress SARS-CoV-2/Flu/RSV. Additional substances have also been previously evaluated on the Xpert Xpress Flu/ RSV assay.

Potentially interfering substances in the nasal passage and nasopharynx may include, but are not limited to: blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms, as well as antibiotics and antivirals. Positive and negative samples were prepared in simulated nasal matrix. Negative samples (N = 8) were tested in the presence of each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (N = 8) were tested per substance with viruses spiked at 3x the analytical LoD determined for each strain. Positive samples tested with the Xpert Xpress SARS-CoV-2/Flu/RSV included one SARS-CoV-2, two influenza A, one influenza B and two RSV (RSV A and RSV B) strains, whereas those tested with the Xpert Xpress Flu/ RSV consisted of six influenza (four influenza A and two influenza B) and four RSV (two RSV A and two RSV B). The substances evaluated are listed in Table 11 with active ingredients and final concentrations tested shown. None of the substances caused interference of the assay performance at the concentrations tested in this study. All positive and negative replicates were correctly identified by the Xpert Xpress SARS-CoV-2/Flu/RSV and/or Xpert Xpress Flu/RSV tests.

Table 11. Potentially Interfering Substances in the Xpert Xpress SARS-CoV-2/Flu/RSV Test and/or Xpert Xpress Flu/RSV Test

Substance/Class	Description/Active Ingredient	Concentration Tested
Control	Simulated nasal matrix	100% (v/v)
Beta-adrenergic bronchodilatora	Albuterol Sulfate	0.83 mg/mL (equivalent to 1 dose per day)
Blood	Blood (Human)	2% (v/v)
BD Universal Transport System	Transport Media	100% (v/v)
Remel M4®	Transport Media	100% (v/v)
Remel M4RT®	Transport Media	100% (v/v)
Remel M5®	Transport Media	100% (v/v)
Remel M6®	Transport Media	100% (v/v)
Throat lozenges, oral anesthetic and analgesic[a]	Benzocaine, Menthol	1.7 mg/mL
Mucin[a]	Purified Mucin protein (Bovine or porcine submaxillary gland)	0.1% (w/v)[b]
Antibiotic, nasal ointment[a]	Mupirocin	10 mg/mL
Saline Nasal Spray[a]	Sodium Chloride (0.65%)	15% (v/v)
Anefrin Nasal Spray	Oxymetazoline, 0.05%	15% (v/v)
PHNY Nasal Drops	Phenylephrine, 0.5%	15% (v/v)
Tamiflu anti-viral drugs[a]	Zanamivir	7.5 mg/mL
Antibacterial, systemic	Tobramycin	4 µg/mL

Substance/Class	Description/Active Ingredient	Concentration Tested
Zicam Nasal Gel	Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum Sulfur	15% (w/v)
Nasal corticosteroid	Fluticasone Propionate	5 µg/mL

[a] Substances/active ingredients and concentrations that were evaluated with the Xpert Xpress SARS-CoV-2/Flu/RSV test.
[b] No interference to the Xpert Xpress Flu/RSV performance observed at a concentration of 2.5%

20.6 Carry-over Contamination

Carry-over studies to establish that single-use, self-contained GeneXpert cartridges prevent carry-over contamination have been conducted for previous Xpert tests developed for the GeneXpert systems, including the Xpert Xpress Flu/RSV. The studies demonstrated that a negative sample when preceded by very a high positive sample in the same GeneXpert module resulted in no carry-over.

21 Reproducibility

The reproducibility of the Xpert Xpress SARS-CoV-2/Flu/RSV test was established at three sites using a 9-member panel including one negative sample, four low positive (~1x LoD) and four moderate positive (~3x LoD) samples. The negative sample consisted of simulated matrix without target microorganism or target RNA. The positive samples were contrived samples in a simulated matrix using inactivated NATtrol SARS-CoV-2 (ZeptoMetrix), cultured viruses Influenza A/California/7/2009, Influenza B/Mass/2/2012, and RSV B/Wash/18537/62.

Testing was conducted over six (6) days, using three (3) lots of Xpert Xpress SARS-CoV-2/Flu/RSV cartridges at three (3) participating sites each with two (2) operators to yield a total of 144 observations per panel member (3 Sites x 2 Operators x 3 Lots x 2 Days/Lot x 2 Runs x 2 Reps = 144 observations/panel member). The results from the study are summarized in Table 12.

Table 12. Summary of Reproducibility Results - % Agreement

Sample	Site 1			Site 2			Site 3			% Total Agreement[a] by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Negative	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
SARS-CoV-2 Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	95.8% (23/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	99.3% (143/144)
SARS-CoV-2 Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (23/23)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
Flu A Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	91.7% (22/24)	91.7% (22/24)	91.7% (44/48)	95.8% (23/24)	100% (24/24)	97.9% (47/48)	96.5% (139/144)
Flu A Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (23/23)	100% (24/24)	100% (47/47)	95.8% (23/24	100% (24/24)	97.9% (47/48)	99.3% (142/143) [b]
Flu B Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
Flu B Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)
RSV Low Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (23/23)	100% (24/24)	100% (47/47)	100% (24/24)	100% (24/24)	100% (48/48)	100% (143/143)
RSV Mod Pos	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (24/24)	100% (24/24)	100% (48/48)	100% (144/144)

[a] Agreement was calculated as the percentage of observed results that were in agreement with the expected results.
[b] Three samples with no valid results (2x non-determinate) [SARS-CoV-2 Mod Pos (1); Flu A Mod Pos (1); RSV Low Pos (1)].

22 References

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23 Cepheid Headquarters Locations

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Telephone: + 33 563 825 300
Fax: + 33 563 825 301
www.cepheidinternational.com

24 Technical Assistance

Before contacting Cepheid Technical Support, collect the following information:

- Product name
- Lot number
- Serial number of the instrument
- Error messages (if any)
- Software version and, if applicable, Computer Service Tag Number

United States

Telephone: + 1 888 838 3222
Email: techsupport@cepheid.com

France

Telephone: +33 563 825 319
Email: support@cepheideurope.com

Contact information for all Cepheid Technical Support offices is available on our website: www.cepheid.com/en/support/contact-us.

25 Table of Symbols

Symbol	Meaning
REF	Catalog number
IVD	*In vitro* diagnostic medical device
	Do not reuse
LOT	Batch code
CE	CE marking – European Conformity
EC REP	Authorized representative in the European Community
	Consult instructions for use
	Caution
	Manufacturer
CC	Country of manufacture
Σ	Contains sufficient for *n* tests
CONTROL	Control
	Expiration date
°C	Temperature limitation
	Biological risks

Cepheid
904 Caribbean Drive
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EC REP

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IVD CE

26 Revision History

Description of Changes: 302-5159, Rev. C to Rev. D

Purpose: Updates to the Instructions for Use

Section	Description of Change
8	Updated information for Sample Collection Kits.
26	Added Revision History table.
Throughout	Updated formatting and layout to current standards.

For Professional Use Only

Allplex™
SARS-CoV-2/FluA/FluB/RSV Assay

(Cat.No. RV10259X)

Multiplex real-time one-step RT-PCR system for detection of SARS-CoV-2, Influenza A virus, Influenza B virus, and Human respiratory syncytial virus from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.

For use with

1. Microlab NIMBUS IVD and Microlab STARlet IVD

2. Seegene NIMBUS and Seegene STARlet

For use with

1. CFX96™ Real-time PCR System (CFX Manager™ Software-IVD v1.6)

2. CFX96™ Dx System (CFX Manager™ Dx Software v3.1)

CE

Σ 100

IVD — **For *in vitro* diagnostic use only**

Seegene Inc.,
Taewon Bldg., 91 Ogeum-ro, Songpa-gu, Seoul, Republic of Korea 05548

EC REP — **Medical Technology Promedt Consulting GmbH**
Altenhofstrasse 80, D-66386 St.Ingbert, Germany

Not available in the U.S.

TABLE OF CONTENTS

NOTICES

- For *in vitro* diagnostic use only.
- The Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay should be performed by qualified, trained personnel.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- **This product is only for use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, and Seegene STARlet in maximum 5 separate runs.**
- **This test has been validated for the following specimen types: nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.** This test has not been validated for any other types of specimens.
- **Store RNA samples at ≤ -20°C until use and keep on ice during use.**
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.
- Workflow in a laboratory should proceed in an unidirectional manner.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.
- Do not pool reagents from different lots or from different tubes of the same lot.
- Do not use the product after its expiry date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive controls. To prevent contamination of reagents, the use of filter-tips is recommended.
- Use separated and segregated working areas for each experiment.

- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separately from the kit's reagents.
- Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components including product residuals and packaging can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiry date is 13 months from the date of manufacture at ≤ -20°C. Please refer to label for the expiry date.
- Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, respectively although the manufacturers are different from each other. Since there are no hardware changes on the devices, the test results are the same for those.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" has been changed to "CFX96™ Dx system". Since there are no hardware changes on the systems, it will be expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD v1.6". The upgraded software includes enhancements to the "Run" menu. These enhancements do not impact the results of data analysis; therefore, the results will be the same.
- This kit is a qualitative *in vitro* test for the single or multiple detection of 3 types of gene (S gene, RdRP gene, and N gene) of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV).

INTENDED USE

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is *in vitro* diagnostic medical device designed for qualitative detection of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) with real-time reverse transcription PCR from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.

PRINCIPLES AND PROCEDURE OVERVIEW

1. Principles

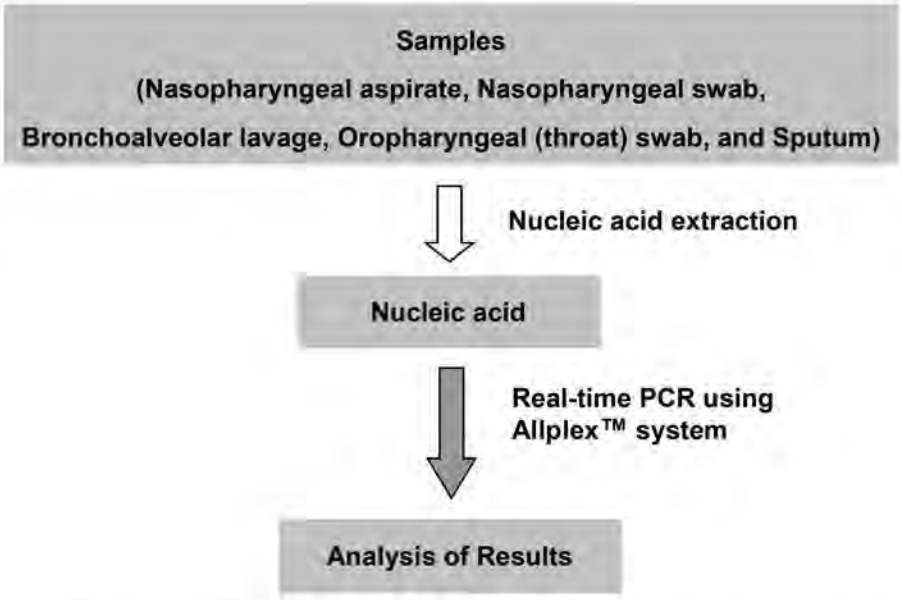
Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is a multiplex real-time PCR assay that enables simultaneous amplification and differentiation of target nucleic acids of S gene, RdRP gene, and N gene of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) with two Internal Controls (Endogenous IC and Exogenous IC).

To perform the multiplex target amplification and detection with superior accuracy in a single reaction, this assay kit employs Seegene's innovative proprietary DPO™, TOCE™ and MuDT™ technologies. The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

Both of endogenous gene and exogenous gene are used as Internal Control (IC). Endogenous IC is used to check the proper sample collection, and Exogenous IC is used to monitor the whole process from nucleic acid extraction and to check for any possible PCR inhibition. Two ICs enable to demonstrate proper sample collection and test validity of each specimen in a single reaction.

To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay. The UDG-dUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.

2. Procedure Overview



< Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay procedure overview >

BACKGROUND INFORMATION

SARS-CoV-2 initially identified in Wuhan, China in December 2019 has spread worldwide and become the coronavirus disease 19 (COVID-19) pandemic. So far, no anti-viral treatment for SARS-CoV-2 is available and the best way to prevent the spreading is to isolate the confirmed patients in quarantine and reduce the number of people in contact. Considering the transmission of SARS-CoV-2 being highly contagious, the foremost concern is to quarantine the infected patients and to distinguish asymptomatic patients before having close contact with people.

The symptoms of COVID-19 are similar to those of respiratory diseases caused by other viruses such as Influenza and difficult to characterize them. To prevent the spreading of SARS-CoV-2 and other respiratory viruses and to treat patients appropriately according to infected virus, it is important to diagnose the viruses accurately, rapidly, and simultaneously.

1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Coronavirus is a RNA virus which contains approximately 27~32 kb of positive single-stranded RNA genome. In human, It causes respiratory tract infections that can range from mild to lethal.

In December 2019, a number of patients with pneumonia of unknown aetiology emerged in Wuhan, China and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus has been identified as the causative agent. Coronavirus disease 19 (COVID-19) has been spreading around the world and the World Health Organization (WHO) has declared COVID-19 pandemic in March 2020. By August 2020, more than 20,000,000 confirmed cases and more than 700,000 deaths of COVID-19 have been reported to the WHO worldwide.

SARS-CoV-2 is transmitted through droplets from infected patients. The COVID-19 infected patients have clinical manifestation which includes the fever and cough as primary clinical presentations and others are shortness of breath and myalgia etc. However, asymptomatic infection cases are also emerging, so it is very important to quickly diagnose and take quarantine measures.

2. Influenza Virus

Influenza virus (family *Orthomyxoviridae*) is an enveloped virus with a single, negative strand RNA genome that is present in 8 separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses and it may contribute to a rapid development of new influenza strains through the interchange of gene segments between different viruses infecting the same cell. There are three types of influenza viruses: A, B, and C. A and B viruses are most common among the three. Especially type A virus is known to be common for infecting not only humans, but also birds and various types of mammals. Among the influenza viruses that invade humans, H1N1 and H3N2 are becoming a problem for infecting children and elder seniors.

3. Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (family *Paramyxoviridae*) is an enveloped virus with a single, negative strand RNA genome. RSV has a high frequency of occurrence among other respiratory diseases. It is categorized as clinically significant for being able to cause death in babies with congenital malformation of cardiac, premature infants, and babies right after open heart surgery. Bronchiolitis is the most common type of RSV infection in infants, but does not appear as often after one year old. On the other hand, pneumonia is a problem for all infants. RSV is responsible for developing 45~75% of bronchiolitis, 15~25% of infant pneumonia, and 6~8% of croup. RSV was originally known as pathogens developing diseases occurring in babies, but recent studies show that RSV occurring in adults can produce results as severe as influenza such as morbidity and leading to deaths. In addition, if patients with declined immune systems (who have gone through bone-marrow transplantation or solid organ transplantation) are infected with RSV that causes pneumonia, the probability of death can rise up to more than 50%.

REAGENTS

The reagents contained in one kit are sufficient for 100 reactions.

Order information (REF Cat. No. RV10259X)

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	SC2FabR MOM	500 µL	Oligo Mix : - Amplification and detection reagent
PREMIX	EM8	500 µL	- RTase - DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	SC2FabR PC	100 µL	Positive Control (PC): - Mixture of pathogen and IC clones
CONTROL IC	RP-V IC 2	1,000 µL	Exogenous Internal Control (IC)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

STORAGE AND HANDLING

All components of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay should be stored at ≤ -20°C. All components are stable under recommended storage conditions until the expiry date stated on the label. The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and Sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Nucleic acid extraction kit (see Nucleic Acid Extraction)
- Clean bench
- Ice Maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white (Cat. No. HSP9655, Bio-Rad)
- Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, barcoded (Cat. No. HSP9955, Bio-Rad)
- Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)[§]
- PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)[§]
- EU 0.1ml 8-tube strip, LP, W, Extra Robust (Cat. No. B72719, BIOplastics)
- EU Optical Wide area 8-Cap Strip (Cat. No. B57801, BIOplastics)
- 96 x 0.1ml Plate, LP, W, FULL, 96 well plate (Cat. No. B70679, BIOplastics)
- Opti-Seal Optical Sealing Sheet (Cat. No. 157300, BIOplastics)

[§] Make sure to use the heat seal and the plate sealer listed above together.

PROTOCOL

1. Specimen Collection, Storage, and Transport

Specimen	SARS-CoV-2	Flu A, Flu B, RSV
Nasopharyngeal aspirate	O	O
Nasopharyngeal swab	O	O
Bronchoalveolar lavage	O	O
Oropharyngeal (throat) swab	O	X
Sputum	O	X

* Flu A, Flu B, and RSV have not been validated with oropharyngeal (throat) swab and sputum specimens.

Note: All samples should be treated as potentially infectious materials. Only permitted are those sample materials, which are collected, transported and stored by attending strictly to the following rules and instructions.
Note: To ensure high quality of samples, samples should be transported as fast as possible at indicated temperature.

A. Specimen Collection

Nasopharyngeal aspirate, Nasopharyngeal swab, Bronchoalveolar lavage, Oropharyngeal (throat) swab

- Obtaining respiratory specimens may be difficult in some patients. In such cases, nasopharyngeal swabs may be collected simply and efficiently.

Manufacturer	Specimen collection device	Cat. No.
COPAN	ESwab	482CE
COPAN	ENAT PM 2ML PERNASAL APPLICATOR	606CS01P*
COPAN	UTM with Flocked Swabs	360C / 305C
DIAGNOSTIC HYBRIDS	UTM with Flexible Minitip Flocked Swab	403C / 406C
COPAN	MSwab® kit	6E012N / 6E013N
COPAN	MSwab® bulk	6E011N
Noble Biosciences	CTM (Clinical Virus Transport Medium)	UTNFS-3B-2-N1P / UTNFS-3B-2
SG Medical	GeneTM Set (GTS2)	T5001
SG Medical	GeneTM Set (GTS1)	T5002

*Please use catalog numbers shown above to purchase products from Seegene Inc.

Sputum

- Give clear instructions to patients when collecting sputum specimens. Patients must either collect samples outside in the open air or away from other people. Patients should not collect samples in confined spaces such as toilets.
- Rinse mouth with water before collecting sputum. The patient should cough deeply and expectorate sputum directly into the container.
- A sputum sample must have a volume of 3~5 mL.

B. Specimen Storage & Transport

Specimen	Storage & Transport		Note
	Temp.	Duration*	
Nasopharyngeal aspirate	2~8°C	3 days	- Performance may be affected by prolonged storage of specimens. - Specimens should also adhere to local and national instructions for transport of pathogenic materials.
Nasopharyngeal swab			
Bronchoalveolar lavage			
Oropharyngeal (throat) swab			
Sputum			

* Duration: The time period from the specimen collection to the final test (includes transport and storage of specimens prior to tests).

2. Nucleic Acid Extraction

[Extraction methods in different specimens]

Note: Please use the automated extraction system according to the specimen shown in the following table.

Specimen	Automated Extraction System					
	Microlab NIMBUS IVD / STARlet IVD		Seegene NIMBUS / STARlet		KingFisher Flex*	MagNA Pure 96*
	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit**	Universal Cartridge Kit	Viral DNA/RNA 200 C Kit**		
Nasopharyngeal aspirate	O	X	O	X	O	O
Nasopharyngeal swab	O	O	O	O	O	O
Bronchoalveolar lavage	O	X	O	X	X	X
Oropharyngeal (throat) swab	O	O	O	O	O	O
Sputum	O	X	O	X	O	O

* Bronchoalveolar lavage has not been validated with KingFisher Flex and MagNA Pure 96.
** Nasopharyngeal aspirate, bronchoalveolar lavage and sputum have not been validated with STARMag 96 X 4 Viral DNA/RNA 200 C Kit.

A. Pre-treatment of specimen

Sputum

- Add 2 volumes of 1X PBS or saline solution to the 1 volume specimen in the 15 mL conical tube and vortex thoroughly to disperse the sample.
- Transfer recommend volume (See Recommended Vol. of 2-C) of sample to a new tube.
- Follow the extraction kit protocol.

Note: In case of the specimens without viscosity, pre- treatment step is NOT required.

B. Internal Control

Note: IC, included in the kit, allows the user not only to verify the nucleic acid extraction procedure, but also to identify any PCR inhibition.

- RP-V IC 2 tube must be loaded on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS or Seegene STARlet before nucleic acid extraction.

- When using KingFisher Flex, MagNA Pure 96, 10 µL of RP-V IC 2 must be added to each specimen before nucleic acid extraction.

C. Automated Nucleic Acid Extraction System

Note: Please use the recommended volumes of specimen and elution as indicated below. For other matters, refer to the manufacturer's manual.

C-1. Microlab NIMBUS IVD

Note: See **Microlab NIMBUS IVD** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab NIMBUS IVD	Hamilton	65415-02*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Please use catalog numbers shown above to purchase products from Seegene Inc.
** Nasopharyngeal aspirate, bronchoalveolar lavage, and sputum have not been validated.

C-2. Microlab STARlet IVD

Note: See **Microlab STARlet IVD** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Microlab STARlet IVD	Hamilton	173000-075*	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit**	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Please use catalog numbers shown above to purchase products from Seegene Inc.
** Nasopharyngeal aspirate, bronchoalveolar lavage, and sputum have not been validated.

C-3. Seegene NIMBUS

Note: See **Seegene NIMBUS** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene NIMBUS	Seegene	65415-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Nasopharyngeal aspirate, bronchoalveolar lavage, and sputum have not been validated.

C-4. Seegene STARlet

Note: See **Seegene STARlet** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
Seegene STARlet	Seegene	67930-03	-
STARMag 96 X 4 Universal Cartridge Kit	Seegene	744300.4. UC384	Specimen: 300 µL Elution: 100 µL
STARMag 96 X 4 Viral DNA/RNA 200 C Kit*	Seegene	EX00013C	Specimen: 300 µL Elution: 100 µL

* Nasopharyngeal aspirate, bronchoalveolar lavage, and sputum have not been validated.

C-5. KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head

Note: See **KingFisher Flex** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
KingFisher™ Flex Purification System, KingFisher with 96 Deep-well Head	Thermo Fisher Scientific	5400630	-
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher Scientific	A42352	Specimen: 200 µL Elution: 80 µL

* Bronchoalveolar lavage has not been validated with KingFisher Flex.

C-6. MagNA Pure 96

Note: See **MagNA Pure 96** operation manual.

Automated Extraction System	Manufacturer	Cat. No.	Recommended Vol.
MagNA Pure 96	Roche Diagnostics	06541089001	-
MagNA Pure 96 DNA and Viral NA Small Volume Kit	Roche Diagnostics	06543588001	Specimen: 200 μL Elution: 100 μL

* Bronchoalveolar lavage has not been validated with MagNA Pure 96.

3. Preparation for Real-time One-step RT-PCR

Note: Correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).

Note: Aerosol resistant filter tips and tight gloves must be used when preparing One-step RT-PCR reactions. Use extreme care to prevent cross-contamination.

Note: Completely thaw all reagents on ice.

Note: Briefly centrifuge reagent tubes to collect residual drops inside of the cap.

Note: The steps A~D are automatically processed on Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet. Refer to each operation manual.

A. Prepare the Reaction Mastermix

5 μL	SC2FabR MOM
5 μL	EM8
10 μL	Total volume of Mastermix

Note: Calculate the total amount of each reagent needed based on the number of reactions including samples and controls.

B. Mix by quick vortexing, and briefly centrifuge.

C. Aliquot 10 μL of Reaction Mastermix into PCR tubes.

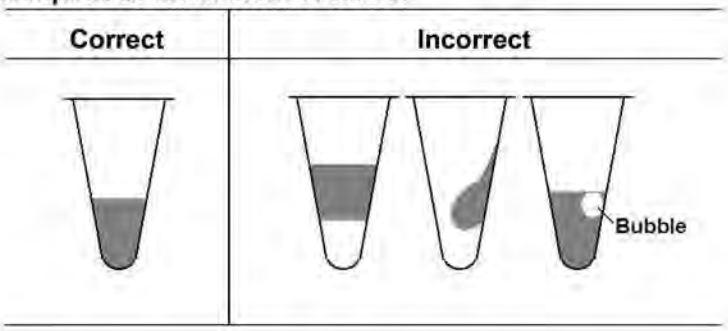
D. Add 10 μL of each sample's nucleic acids into the tube containing Reaction Mastermix.

10 μL	Reaction Mastermix
10 μL	Sample's nucleic acid
20 μL	Total volume of reaction

E. Close and briefly centrifuge the PCR tubes.

F. Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm for a longer time.

Note: It is recommended to centrifuge PCR tubes before PCR to eliminate air bubbles and collect all residual liquids at the bottom of tubes.



Note: Use a new sterile pipette tip for each sample.

Note: For **Negative Control (NC)**, use 10 μL of "**RNase-free Water**" instead of sample's nucleic acid.

Note: For **Positive Control (PC)**, use 10 μL of "**SC2FabR PC**" instead of sample's nucleic acid.

Note: Be careful not to cross-contaminate the Reaction Mastermix and samples with the Positive Control.

Note: Do not label the reaction tube on its cap. Fluorescence is detected from the top of each reaction tube.

REAL-TIME PCR INSTRUMENT SET UP AND RESULT ANALYSIS

1. CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6)

1.1. Real-time PCR Instrument Setup

Note: CFX96™ Real-time PCR Detection System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start run.

A. Protocol Setup

1) In the main menu, select **"File"** → **"New"** → **"Protocol"** to open **"Protocol Editor"**.

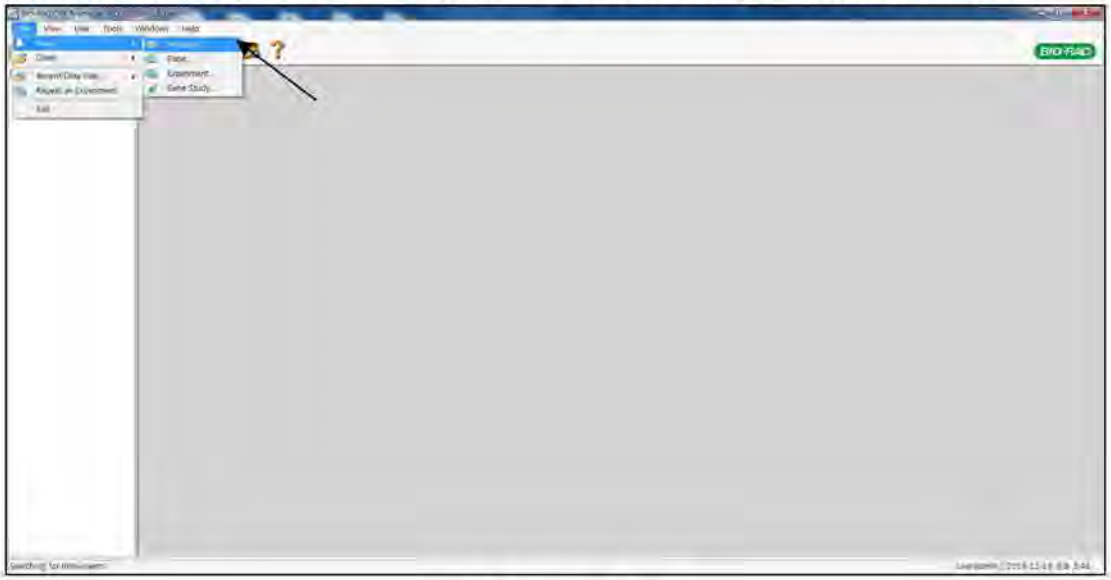


Fig. 1. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	3	95°C	10 sec
4		60°C	40 sec
5		72°C	20 sec
6	GOTO Step 3, 2 more times		
7	42	95°C	10 sec
8*		60°C	15 sec
9*		72°C	10 sec
10	GOTO Step 7, 41 more times		

Note*: Plate Read Step. Fluorescence is detected at 60°C and 72°C (Step 8 and 9).

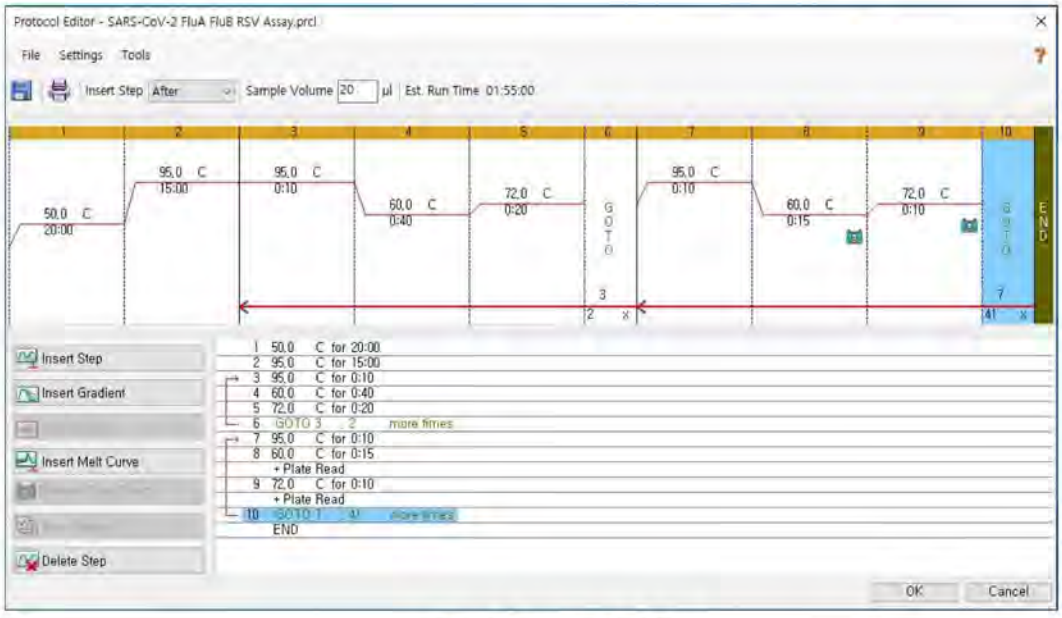


Fig. 2. **Protocol Editor**

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click **"OK"** and save the protocol to open the **"Experiment Setup"** window.

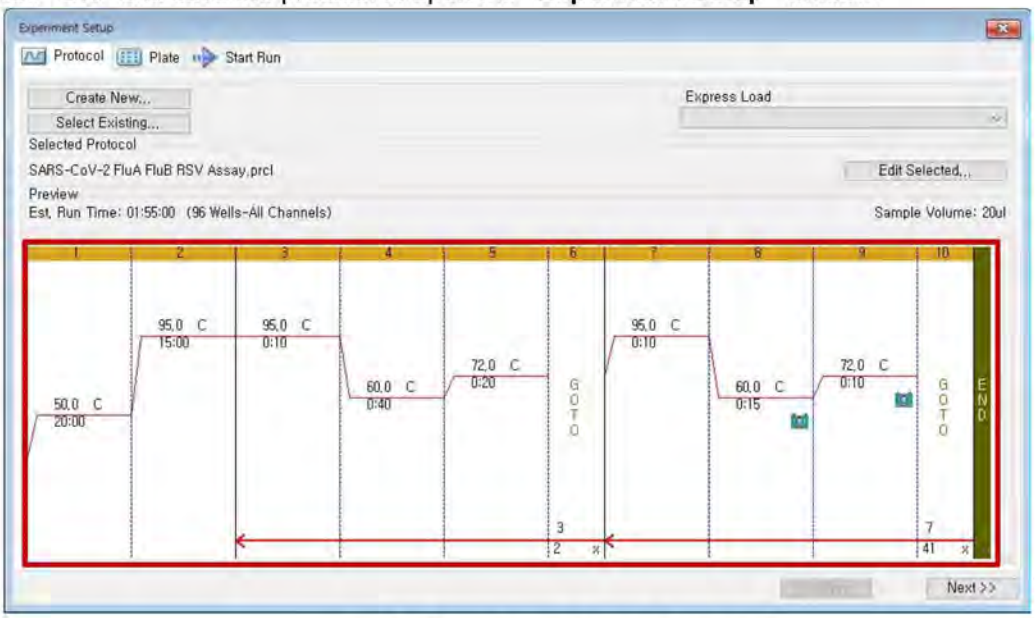


Fig. 3. **Experiment Setup: Protocol**

B. Plate Setup

1) From **"Plate"** tab in **"Experiment Setup"**, click **"Create New"** to open **"Plate Editor"** window.

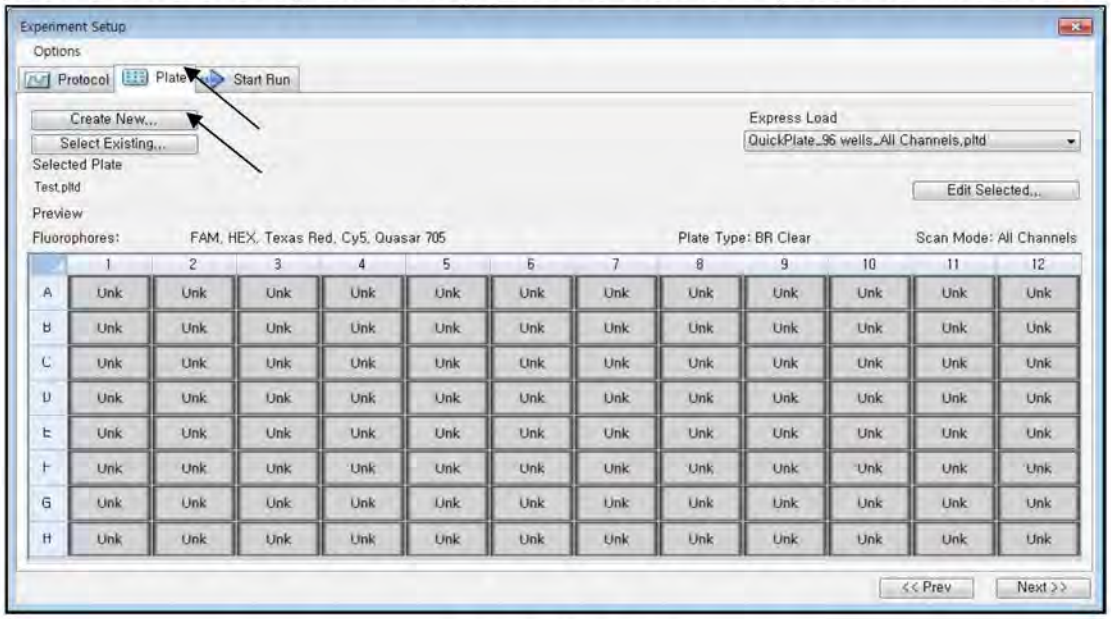


Fig. 4. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM, HEX, Cal Red 610, Quasar 670***) that will be used and click **"OK"**.

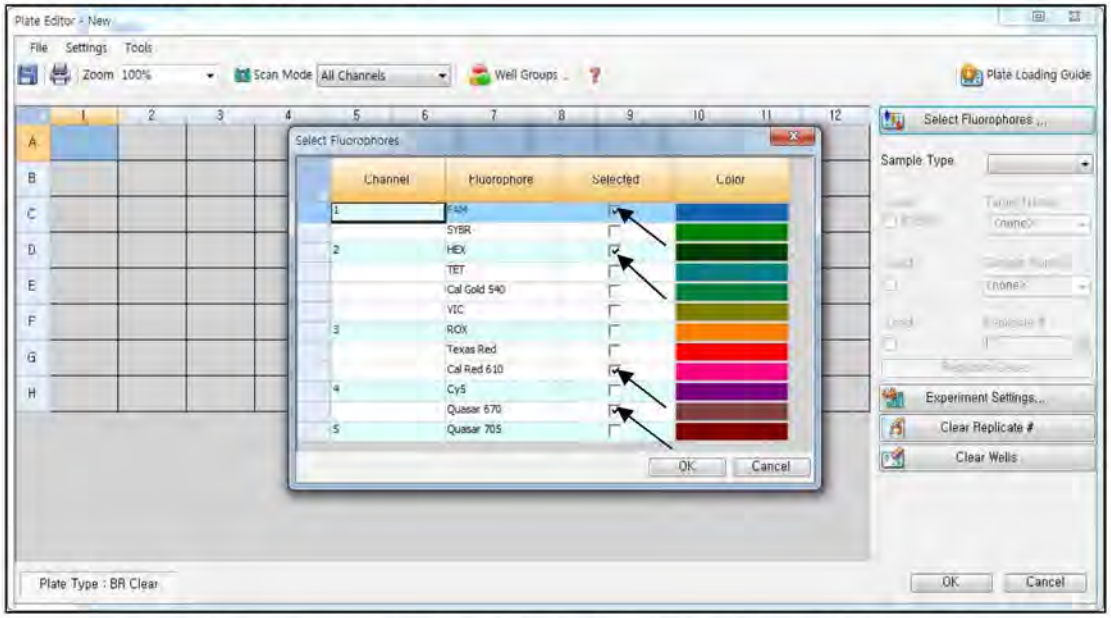


Fig. 5. **Select Fluorophores** (***FAM, HEX, Cal Red 610,*** *and* ***Quasar 670***)

3) Select the wells where the PCR tube will be placed and select their sample types from the **"Sample Type"** drop-down menu.

- ***Unknown****: Clinical samples*
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM, HEX, Cal Red 610, and Quasar 670***) to specify the fluorophores to be detected in the selected wells.

5) Type **"Sample Name"** and press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (*96 wells*)** and **"Plate Type" (*BR White*).**

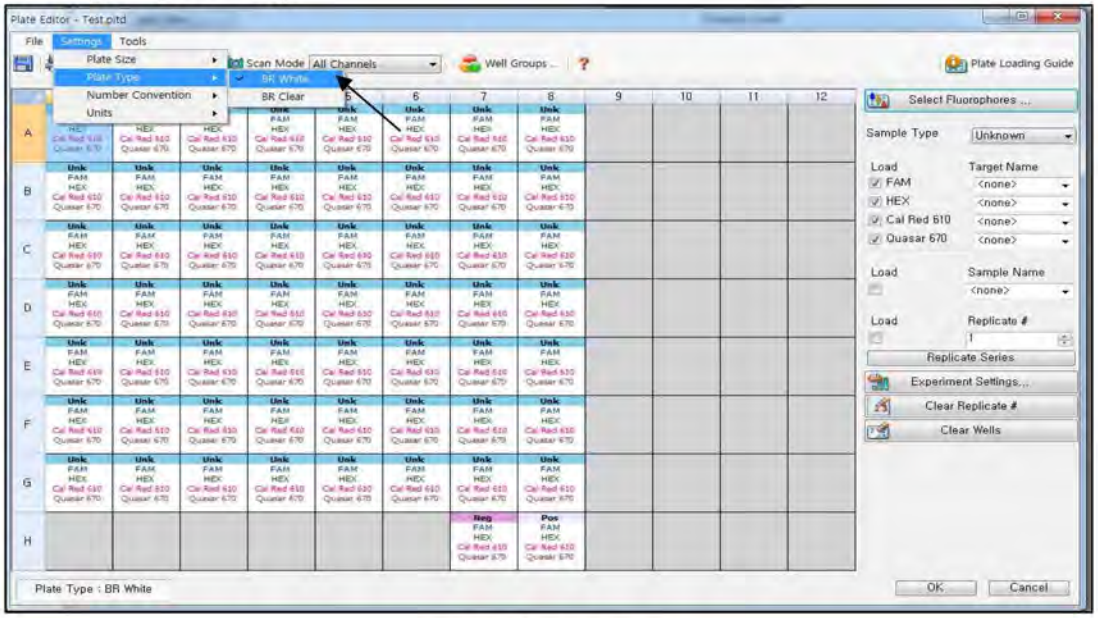


Fig. 6**. Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Experiment Setup"** window.

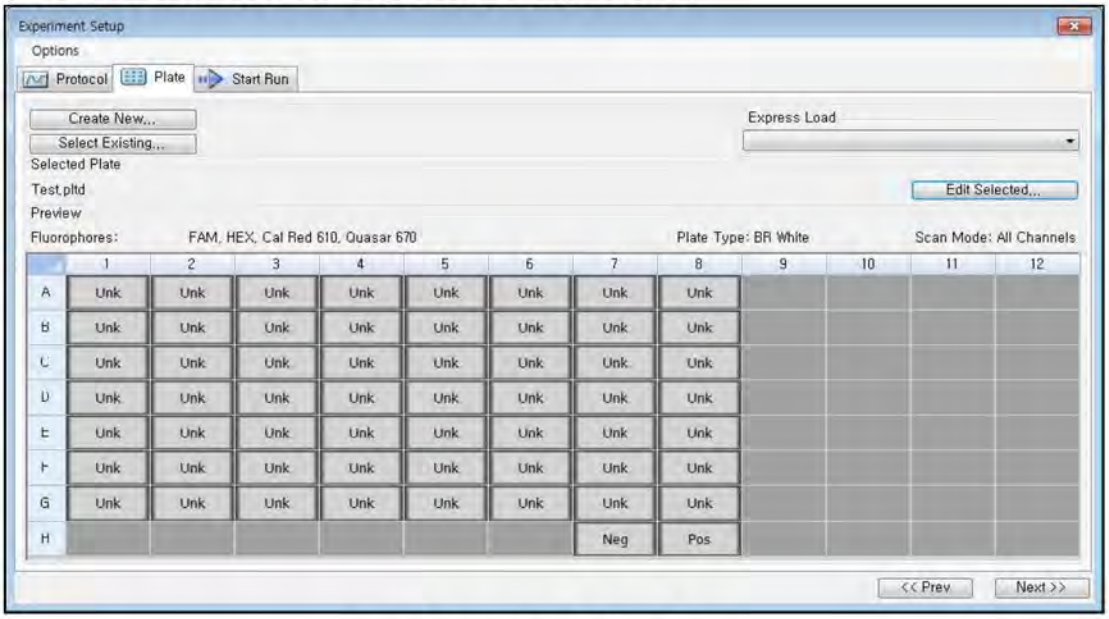


Fig. 7**. Experiment Setup: Plate**

9) Click **"Next"** to Start Run.

C. Start Run

1) From **"Start Run"** tab in **"Experiment Setup"**, click **"Close Lid"** to close the instrument lid.

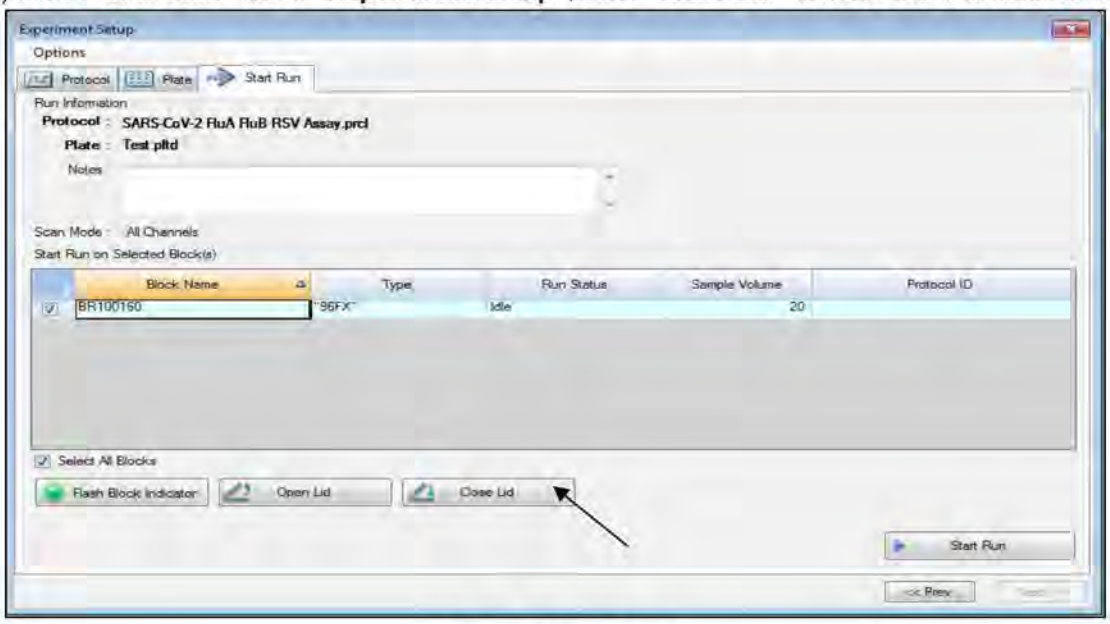


Fig. 8. **"Close Lid"**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

1.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantitation"** tab to see the amplification curve results.

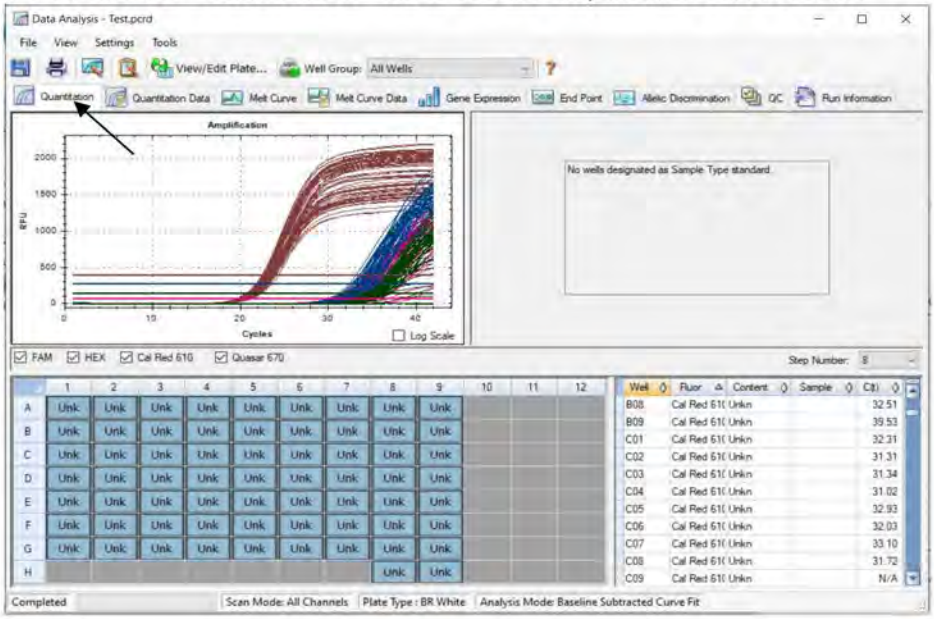


Fig. 9. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Analysis Mode of Settings menu.

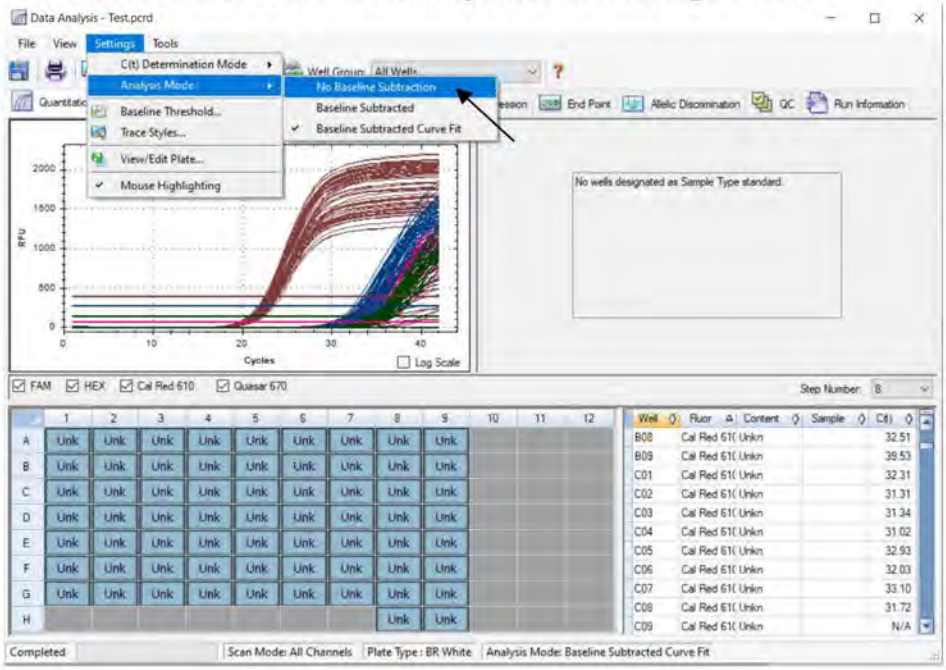


Fig. 10. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Tools menu.

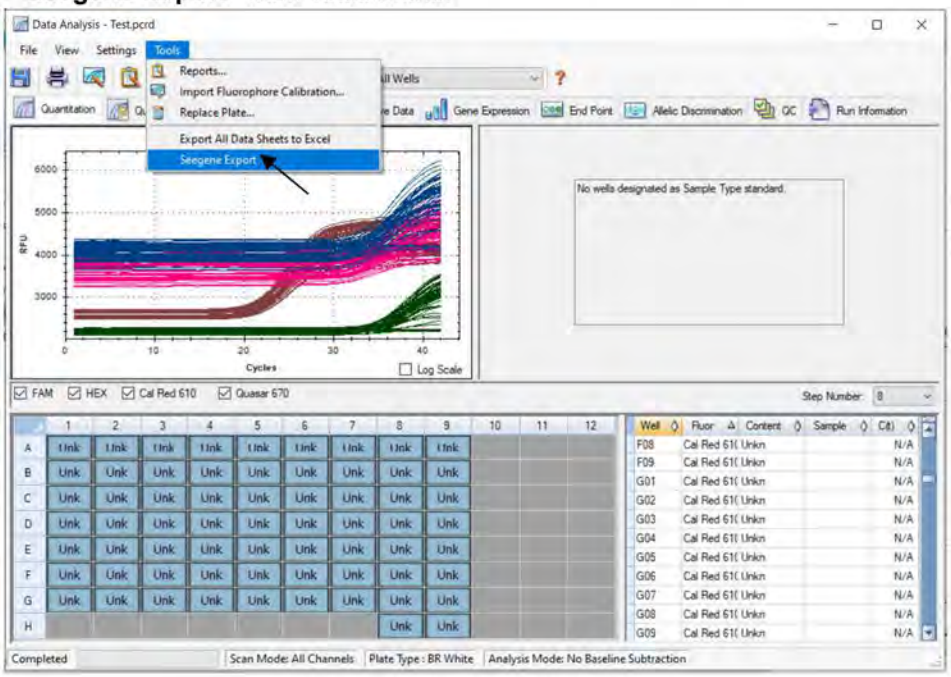


Fig. 11. **Seegene Export**

4) Choose a location to save data and click **"OK"**.

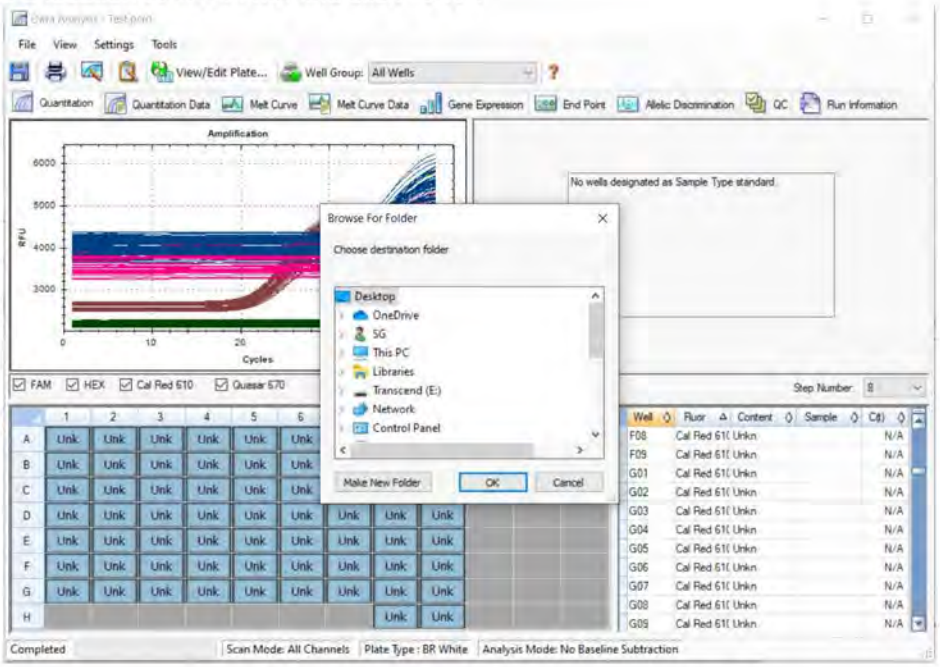


Fig. 12. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program, and click **"Option"** to select ***CFX96 or CFX96 Dx*** in the **"Instrument"**.

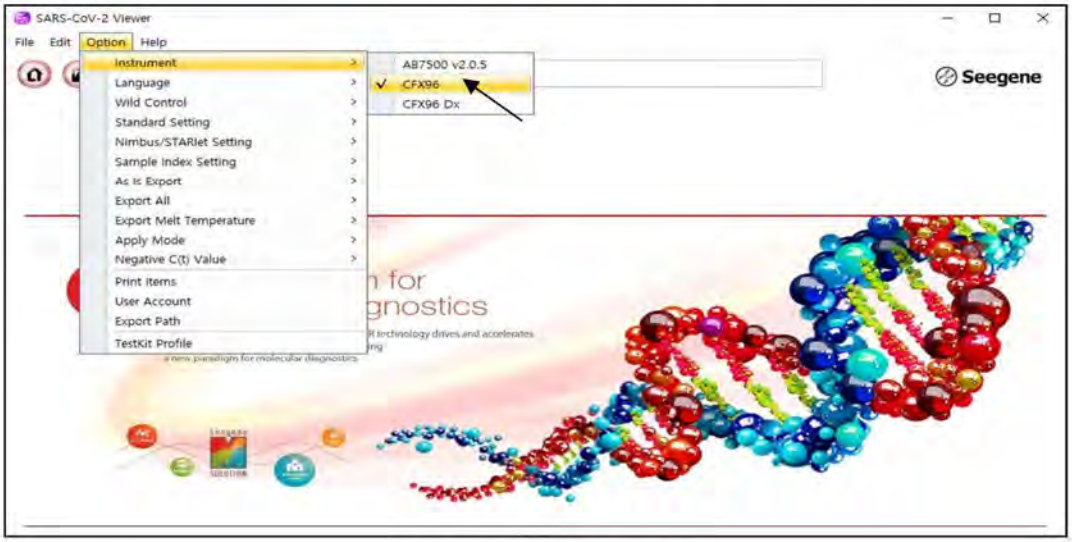


Fig. 13. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep8", open the results file, and select the test kit from the **"PRODUCT"** menu.

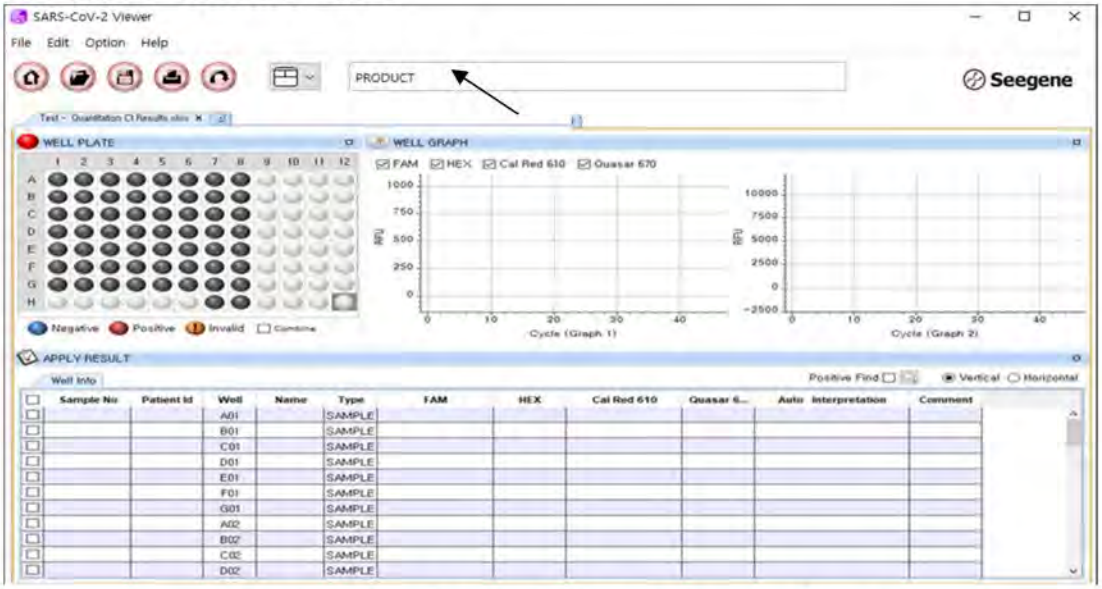


Fig. 14. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

Fig. 15. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To check the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result								
	FAM (C_t)		HEX (C_t)		Cal Red 610 (C_t)		Quasar670 (C_t)		Auto Interpretation
	S gene	RSV	RdRP gene	Flu B	N gene	Flu A	Endo IC	Exo IC	
SC2FabR Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

2. CFX96™ Dx System (CFX Manager™ Dx v3.1)

2.1 Real-time PCR Instrument Setup

Note: CFX96™ Dx System (Bio-Rad) experiment setup can be divided into three steps: Protocol Setup, Plate Setup, and Start Run.

A. Protocol Setup

1) In the main menu, select **"File"** → **"New"** → **"Protocol"** to open **"Protocol Editor"**.

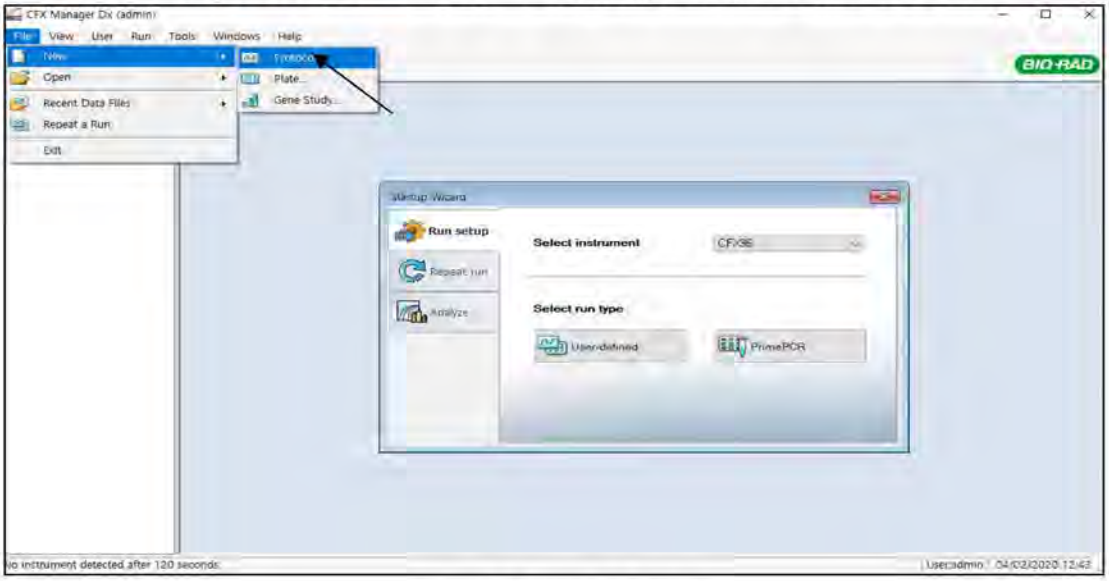


Fig. 16. **Protocol Setup**

2) In **"Protocol Editor"**, define the thermal profile as follows:

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	3	95°C	10 sec
4		60°C	40 sec
5		72°C	20 sec
6	GOTO Step 3, 2 more times		
7	42	95°C	10 sec
8*		60°C	15 sec
9*		72°C	10 sec
10	GOTO Step 7, 41 more times		

Note*: Plate Read Step. Fluorescence is detected at 60°C and 72°C (Step 8 and 9).

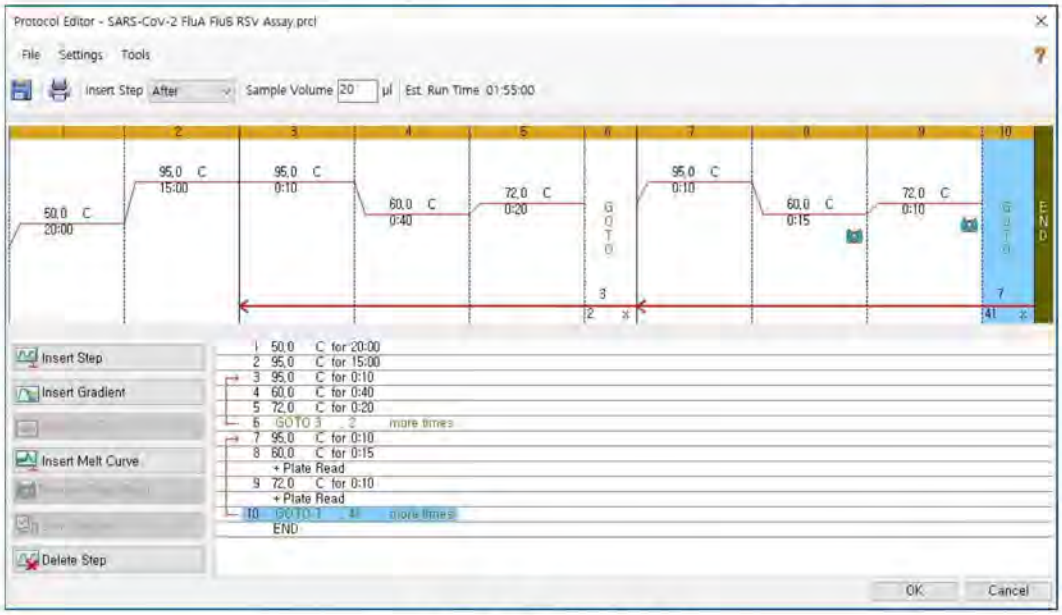


Fig. 17. **Protocol Editor**

3) Click the box next to **"Sample Volume"** to directly input 20 µL.

4) Click **"OK"** and save the protocol to open the **"Run Setup"** window.

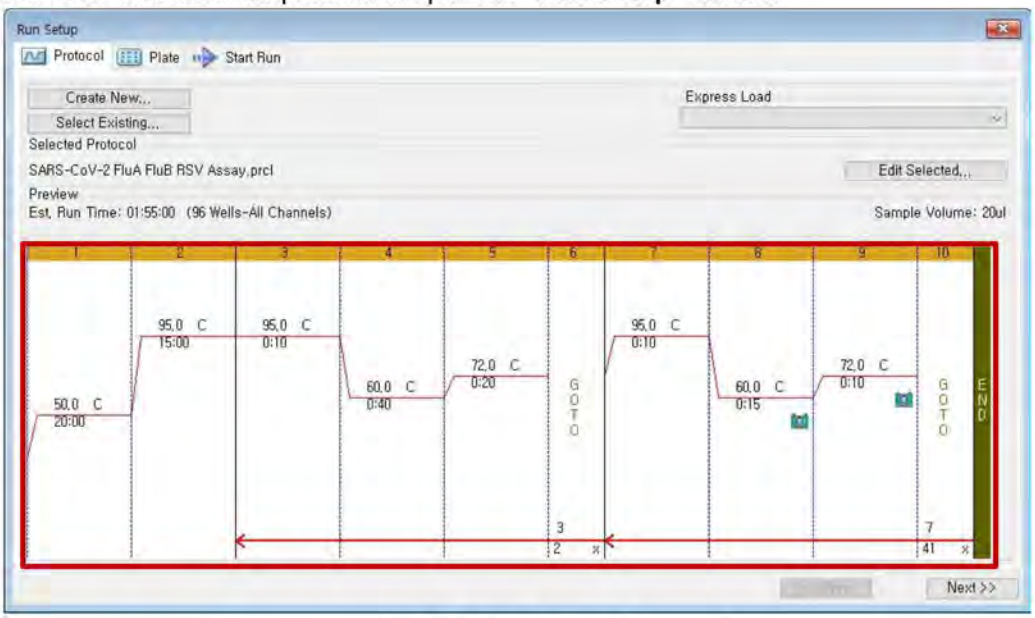


Fig. 18. **Run Setup: Protocol**

B. Plate Setup

1) From **"Plate"** tab in **"Run Setup"**, click **"Create New"** to open **"Plate Editor"** window.

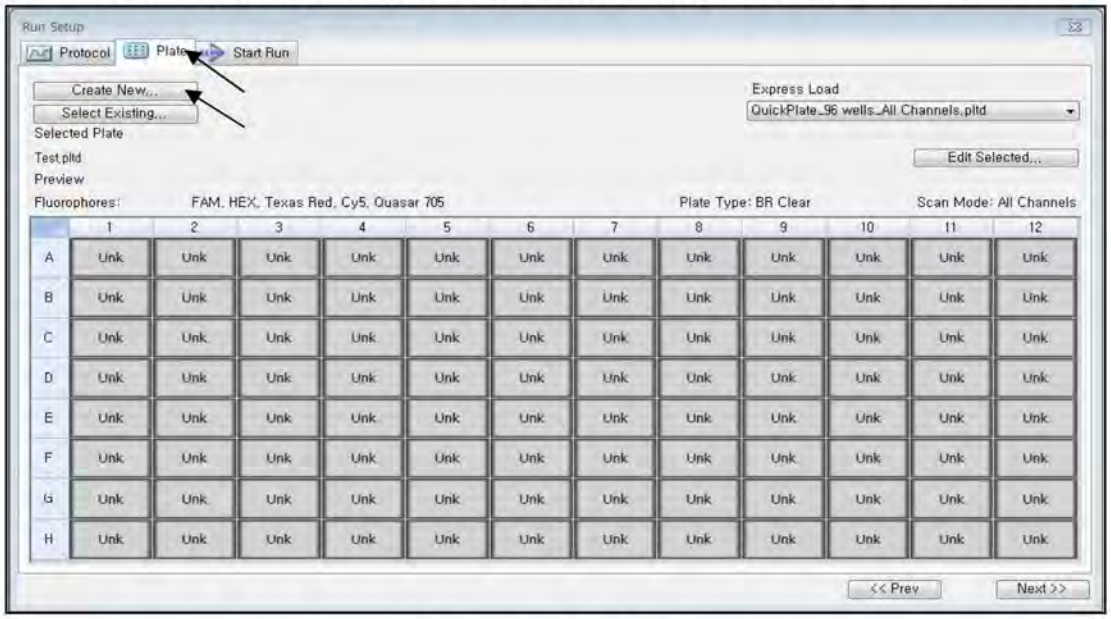


Fig. 19. **Plate Editor**

2) Click **"Select Fluorophores"** to indicate the fluorophores (***FAM, HEX, Cal Red 610, Quasar 670***) that will be used and click **"OK"**.

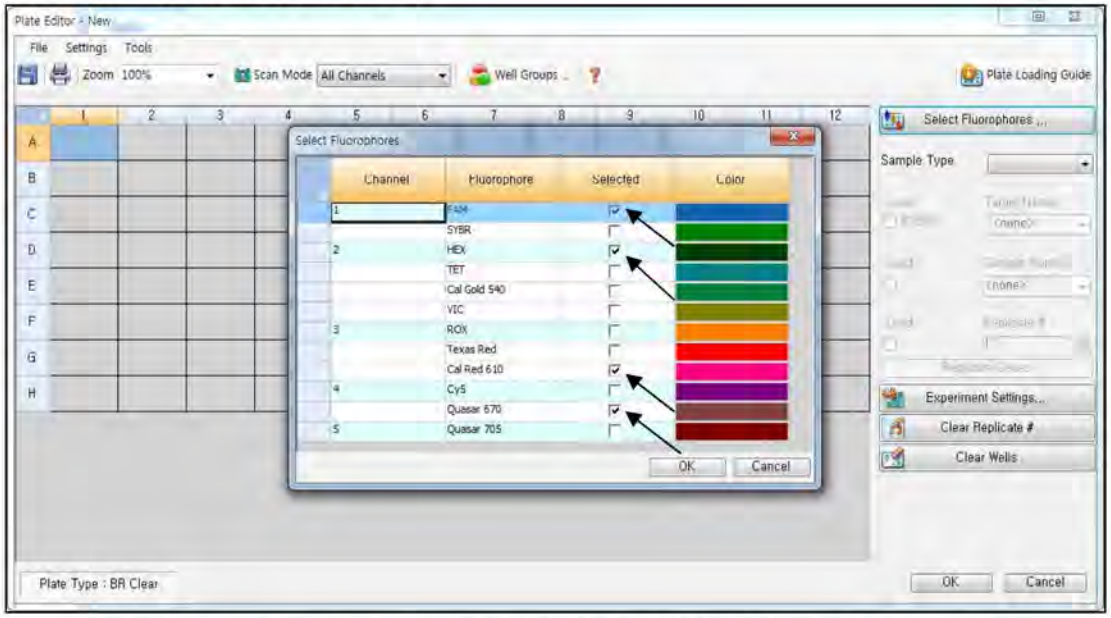


Fig. 20. **"Select Fluorophores"** (***FAM, HEX, Cal Red 610,*** *and* ***Quasar 670***)

3) Select the wells where the PCR tube will be placed and select their sample types from the **"Sample Type"** drop-down menu.

- ***Unknown****: Clinical samples*
- ***Negative Control***
- ***Positive Control***

4) Click on the appropriate checkboxes (***FAM, HEX, Cal Red 610, and Quasar 670***) to specify the fluorophores to be detected in the selected wells.

5) Type **"Sample Name"** and press enter key.

6) In **"Settings"** of the **"Plate Editor"** main menu, choose the **"Plate Size" (*96 wells*)** and **"Plate Type" (*BR White*).**

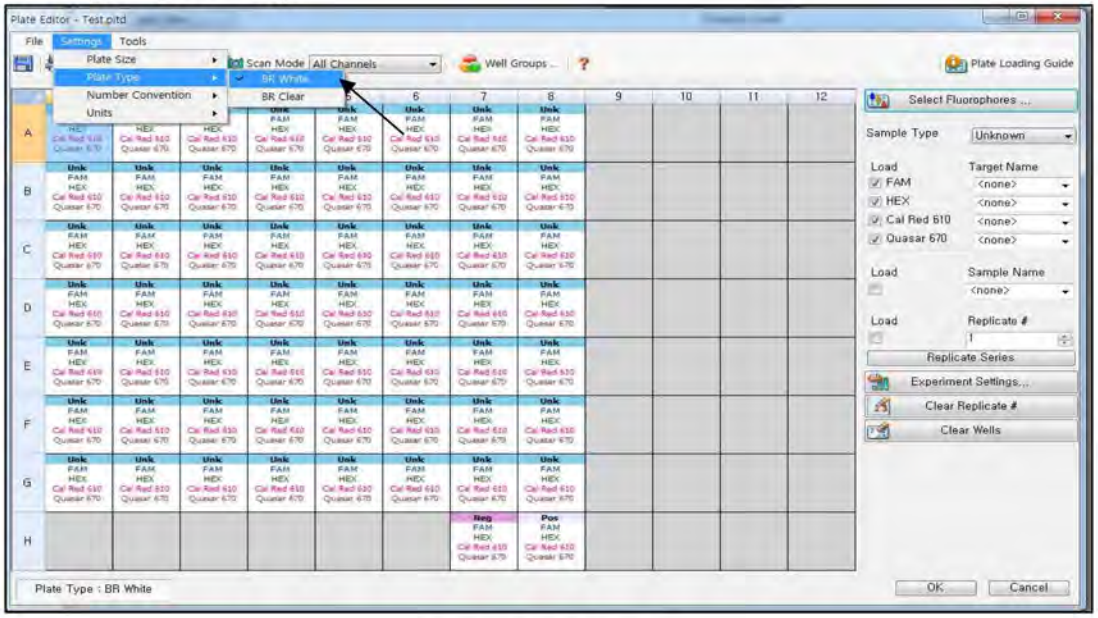


Fig. 21**. Plate Setup**

7) Click **"OK"** to save the new plate.

8) You will be returned to the **"Run Setup"** window.

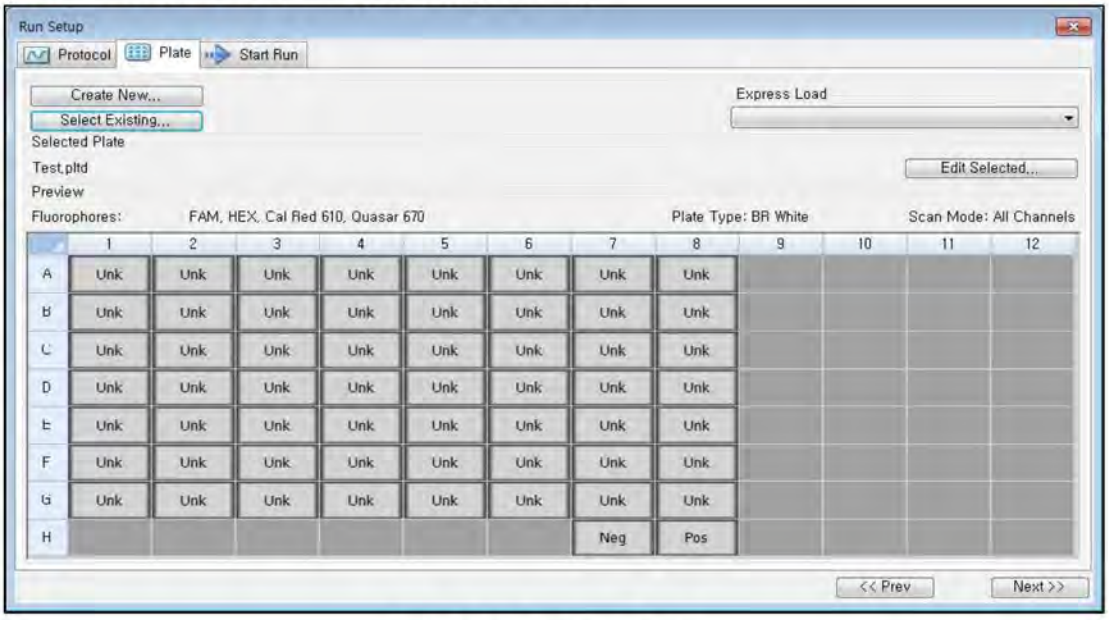


Fig. 22**. Run Setup: Plate**

9) Click **"Next"** to Start Run.

C. Start Run

1) From **"Start Run"** tab in **"Run Setup"**, click **"Close Lid"** to close the instrument lid.

Fig. 23. **Close Lid**

2) Click **"Start Run"**.

3) Store the run file either in My Documents or in a designated folder. Input the file name, click **"SAVE"**, and the run will start.

2.2. Data Analysis

A. Create folders for data export

1) To save data of all detection steps of amplification curves from the result file, create one folder.

2) Folder name may be as desired by user (For 'Seegene Export' function, folders "QuantStep8" and "QuantStep9" are automatically created to save each amplification curve data under the folder created by user).

B. Pre-settings for Data Analysis in CFX96™

1) After the test, click the **"Quantification"** tab to see the amplification curve results.

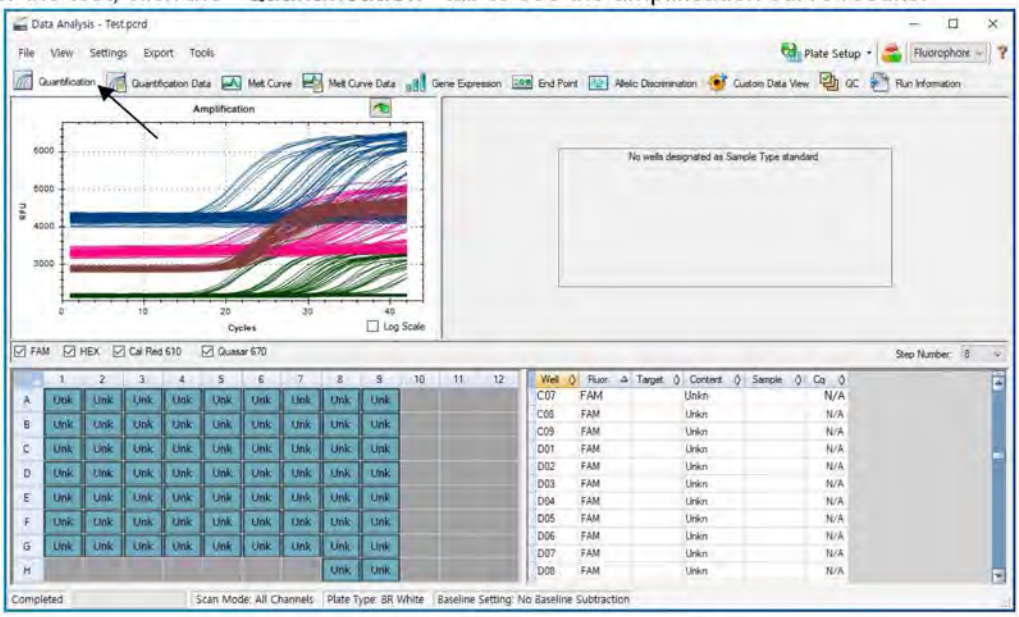


Fig. 24. **Amplification curve results**

2) Select **"No Baseline Subtraction"** from Baseline Setting of Settings menu.

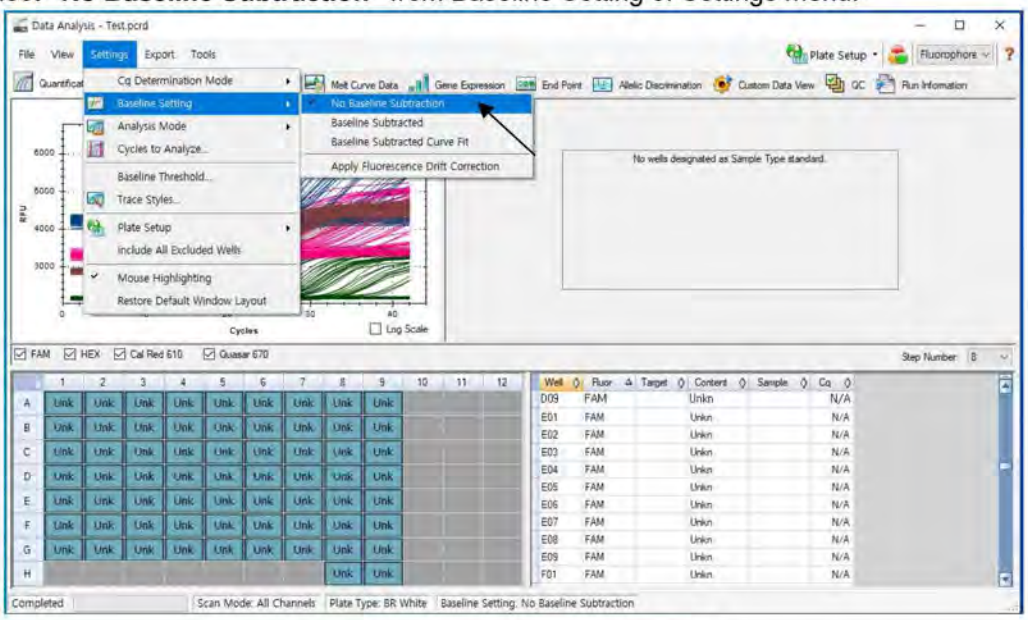


Fig. 25. **No Baseline Subtraction**

3) Select **"Seegene Export"** from Export menu.

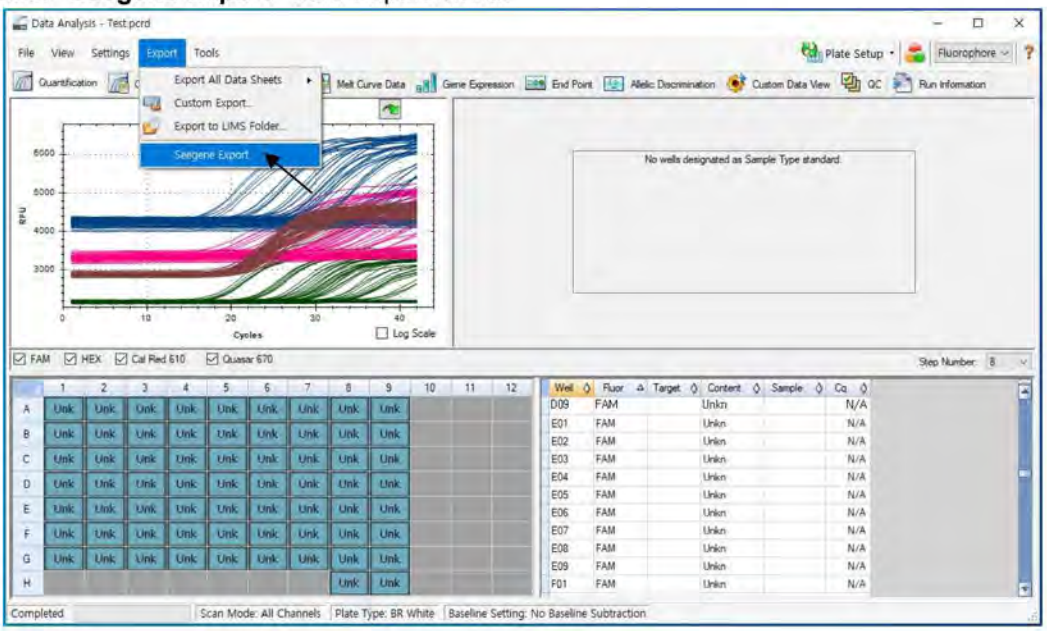


Fig. 26. **"Seegene Export"**

4) Choose a location to save data and click **"OK"**.

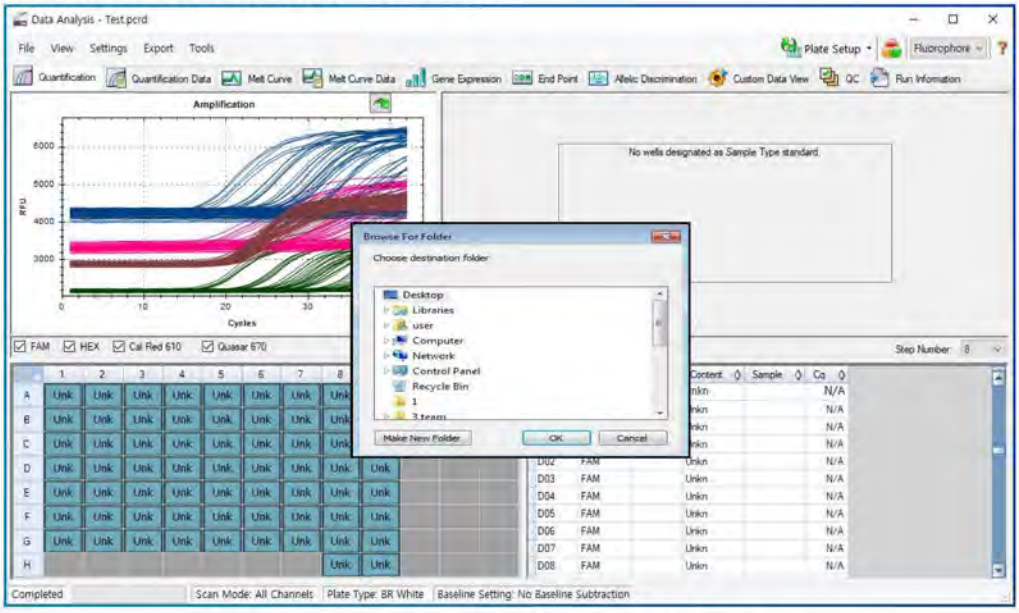


Fig. 27. **Seegene Export to designated folder**

C. Settings for Data Analysis in Seegene Viewer

1) Open Seegene Viewer program, and click **"Option"** to select ***CFX96 or CFX96 Dx*** in the **"Instrument"**.

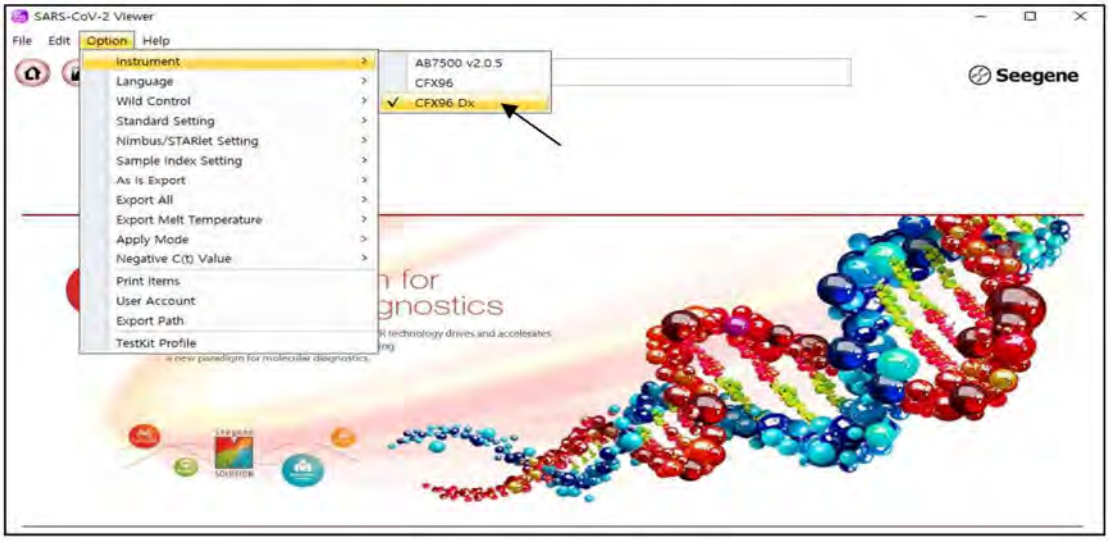


Fig. 28. **Seegene Viewer**

2) Click **"Open"** to find the saved file in folder "QuantStep8", open the results file, and select the test kit from the **"PRODUCT"** menu.

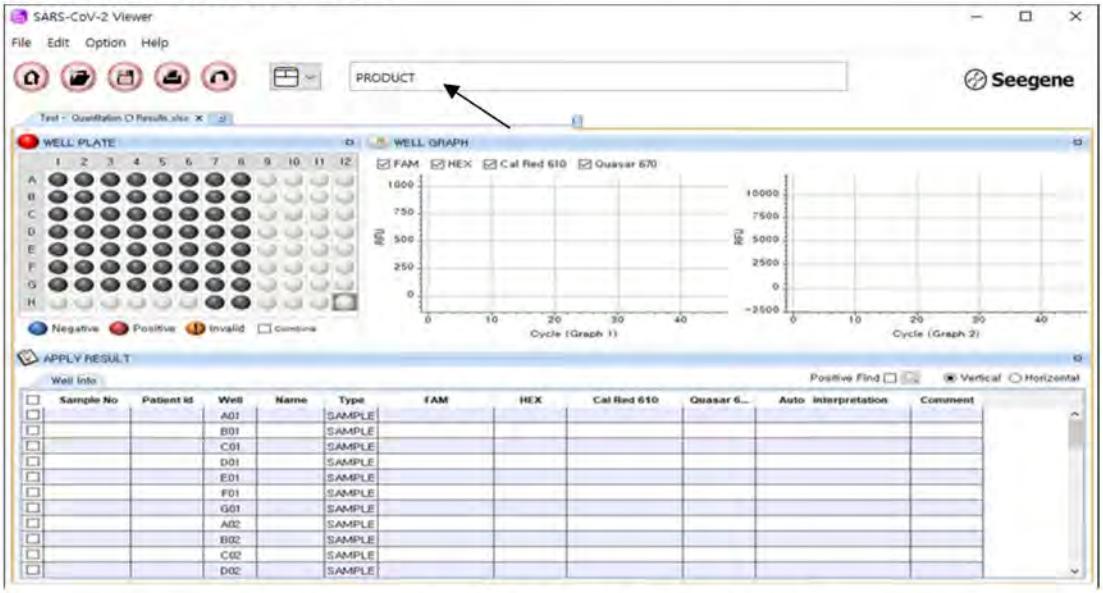


Fig. 29. **Settings for Data Analysis in Seegene Viewer**

3) Check the result for each well.

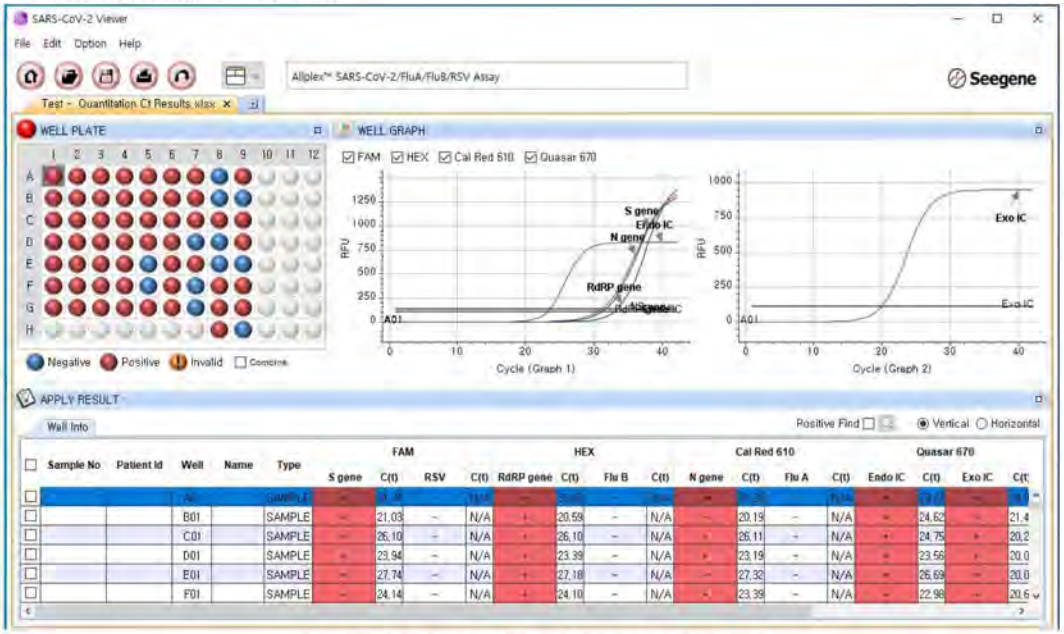


Fig. 30. **Test result on Seegene Viewer**

4) Validity Criteria of Control Results

a. Valid Assay Run

To check the validity of experiments, the PCR runs should be accompanied with PC (Positive Control) and NC (Negative Control). Assay run is determined as valid when all of the following criteria are met:

Control	Seegene Viewer Result								
	FAM (C_t)		HEX (C_t)		Cal Red 610 (C_t)		Quasar670 (C_t)		Auto Interpretation
	S gene	RSV	RdRP gene	Flu B	N gene	Flu A	Endo IC	Exo IC	
SC2FabR Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

b. Invalid Assay Run

In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

RESULTS

1. Analyte Information

Fluorophores	Analytes	
	Graph 1	Graph 2
FAM	S gene	RSV
HEX	RdRP gene	Flu B
Cal Red 610	N gene	Flu A
Quasar 670	Endo IC	Exo IC

2. Interpretation of Results

Analytes	C_t value	Result
Targets	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)
IC	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)

2-1. Interpretation of SARS-CoV-2

S gene	RdRP gene	N gene	Interpretation of SARS-CoV-2
+	+	+	SARS-CoV-2 RNA, Detected
+	+	-	
+	-	+	
-	+	+	
+	-	-	
-	+	-	
-	-	+	
-	-	-	SARS-CoV-2 RNA, Not detected

2-2. Overall Interpretation

Target Result	Endo IC Result	Exo IC Result	Overall Interpretation
+	+	+	Target Nucleic acid, Detected
	-	+	
	+	-	
-	+	+	Target Nucleic acid, Not detected
	-	+	Invalid [1]
	+	-	Invalid [2]
+/-	-	-	Invalid [3]

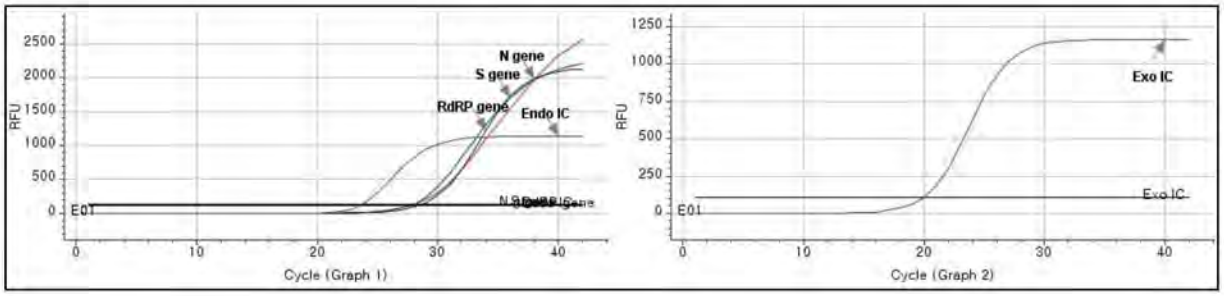
1) Sample collection could be incorrect. Repeat from the sample collection. See TROUBLESHOOTINGS section for the detailed instruction.

2) Extraction or PCR could be inhibited. Repeat from the nucleic acid extraction. See TROUBLESHOOTINGS section for the detailed instruction.

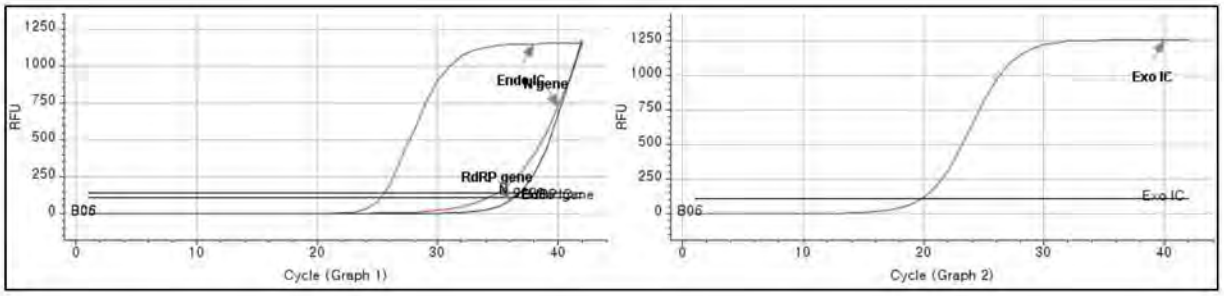
3) Repeat from the nucleic acid extraction. If the same result is shown, repeat from the sample collection. See TROUBLESHOOTINGS section for the detailed instruction.

3. Application to Clinical Samples

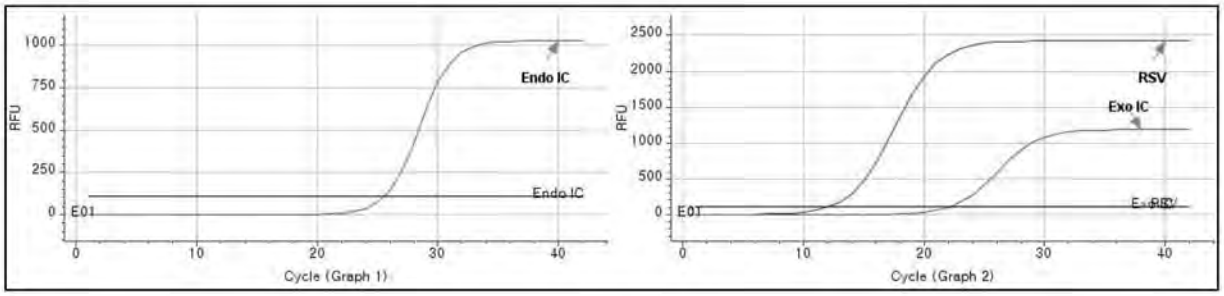
Clinical Sample 1



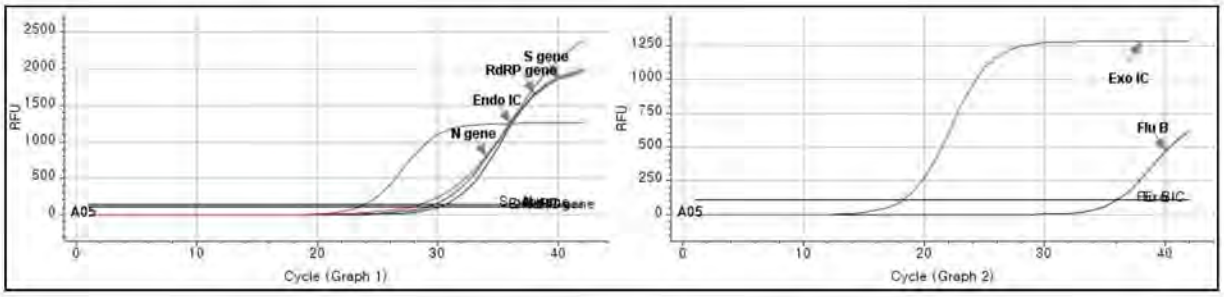
Clinical Sample 2



Clinical Sample 3



Clinical Sample 4



Sample	Seegene Viewer Result (C_t)								Auto Interpretation
	FAM		HEX		Cal Red 610		Quasar 670		
	S gene	RSV	RdRP gene	Flu B	N gene	Flu A	Endo IC	Exo IC	
1	28.06	N/A	28.33	N/A	28.11	N/A	23.49	20.01	SARS-CoV-2
2	N/A	N/A	36.41	N/A	34.99	N/A	25.38	19.90	SARS-CoV-2
3	N/A	12.08	N/A	N/A	N/A	N/A	25.60	22.16	RSV
4	29.73	N/A	30.18	36.07	28.55	N/A	23.58	18.29	SARS-CoV-2,Flu B

TROUBLESHOOTING

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 8) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	If IC had been exogenously added to the specimen prior to extraction, the absence of IC signal may indicate loss of nucleic acids during the extraction. Make sure to use recommended extraction method. If due to inhibitors, re-extract the original specimen or the specimen may be diluted with saline buffer 1/3~1/10 fold and then add "RP-V IC2" to the diluted specimen.
No Exogenous Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not Exo IC, then Exo IC amplification may have been inhibited by high titer of target pathogen. If you want to observe Exo IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from RT-PCR step. If the same result is shown, the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No Endogenous Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not Endo IC, then Endo IC amplification may have been inhibited by high titer of target pathogen. If you want to observe Endo IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from RT-PCR step. If the same result is shown, the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect specimen collection	Please collect the specimen again and repeat the test from extraction step.
Putative false positive or target signal(s) observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
Putative False negative or no signal observed in Positive Control	Error in specimen collection	Please check the specimen collection method, and re-collect the specimen.
	Incorrect storage of the specimen	Please re-collect the specimen and repeat the entire procedure. Ensure that the specimen is stored as recommended.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid.
	Error in adding nucleic acid to correct PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and carefully repeat the test if necessary.
	Presence of inhibitor	Please dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect PCR mixture	Confirm that all components are added to the reaction mixture (Sensitivity is compromised with pre-composed premix). All reagents must be homogenized and spun down before use.
Spikes in any cycles of amplification curve	Bubble in the PCR tube	Centrifuge the PCR tube before run.

PERFORMANCE

1. Analytical Specificity

The high specificity of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is ensured by the oligos designed specifically for the targets of interest. Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was tested for cross-reactivity to 120 different pathogens, and PCR amplification and detection were only identified for the specified targets.

NO.	Organism	Source	Isolate No.	Result†
1	Influenza A H1N1 (Brisbane/59/07)	ZMC	0810244CF	Flu A Detected
2	Influenza A H1N1 (New Cal/20/99)	ZMC	0810036CF	Flu A Detected
3	Influenza A H1N1 (PR/8/34)	ZMC	0810245CF	Flu A Detected
4	Influenza A H1N1 (Singapore/63/04)	ZMC	0810246CF	Flu A Detected
5	Influenza A H1N1 (Solomon Islands/03/06)	ZMC	0810036CFN	Flu A Detected
6	Influenza A H1N1 (Taiwan/42/06)	ZMC	0810247CF	Flu A Detected
7	Influenza A virus (H1N1) (A/FM/1/47)	ATCC	VR-97	Flu A Detected
8	Influenza A virus (H1N1) (A/NWS/33)	ATCC	VR-219	Flu A Detected
9	Influenza A virus (H1N1) (A/WS/33)	ATCC	VR-825	Flu A Detected
10	Influenza A H1N1pdm (California/07/09)	ZMC	0810165CF	Flu A Detected
11	Influenza A H1N1pdm (Canada/6294/09)	ZMC	0810109CFJ	Flu A Detected
12	Influenza A H1N1pdm (Mexico/4108/09)	ZMC	0810166CF	Flu A Detected
13	Influenza A H1N1pdm (NY/01/09)	ZMC	0810248CF	Flu A Detected
14	Influenza A H1N1pdm (NY/02/09)	ZMC	0810109CFN	Flu A Detected
15	Influenza A H1N1pdm (NY/03/09)	ZMC	0810249CF	Flu A Detected
16	Influenza A H1N1pdm Virus (Michigan/45/15)	ZMC	0810538CF	Flu A Detected
17	Influenza A virus (H1N1-pdm09) (A/Virginia/ATCC1/2009)	ATCC	VR-1736	Flu A Detected
18	Influenza A H3N2 (Brisbane/10/07)	ZMC	0810138CF	Flu A Detected
19	Influenza A H3N2 (HK/8/68)	ZMC	0810250CF	Flu A Detected
20	Influenza A H3N2 (Perth/16/09)	ZMC	0810251CF	Flu A Detected
21	Influenza A H3N2 (Texas/50/12)	ZMC	0810238CF	Flu A Detected
22	Influenza A H3N2 (Victoria/361/11)	ZMC	0810240CF	Flu A Detected
23	Influenza A H3N2 (Wisconsin/67/05)	ZMC	0810252CF	Flu A Detected
24	Influenza A H3N2 Virus (Hong Kong/4801/14)	ZMC	0810526CF	Flu A Detected
25	Influenza A virus (H3N2) (A/Aichi/2/68)	ATCC	VR-547	Flu A Detected

NO.	Organism	Source	Isolate No.	Result†
26	Influenza A virus (H3N2) (A/Port Chalmers/1/73)	ATCC	VR-810	Flu A Detected
27	Influenza B (Brisbane/33/08)	ZMC	0810253CF	Flu B Detected
28	Influenza B (Brisbane/60/08)	ZMC	0810254CF	Flu B Detected
29	Influenza B (Florida/02/06)	ZMC	0810037CF	Flu B Detected
30	Influenza B (Florida/04/06)	ZMC	0810255CF	Flu B Detected
31	Influenza B (Florida/07/04)	ZMC	0810256CF	Flu B Detected
32	Influenza B (Lee/40)	ZMC	0810257CF	Flu B Detected
33	Influenza B (Malaysia/2506/04)	ZMC	0810258CF	Flu B Detected
34	Influenza B (Massachusetts/2/12)	ZMC	0810239CF	Flu B Detected
35	Influenza B (Panama/45/90)	ZMC	0810259CF	Flu B Detected
36	Influenza B (Texas/6/11)	ZMC	0810242CF	Flu B Detected
37	Influenza B (Wisconsin/1/10)	ZMC	0810241CF	Flu B Detected
38	Respiratory Syncytial Virus Type A (12/2014 Isolate #2)	ZMC	0810452CF	RSV Detected
39	Respiratory Syncytial Virus Type A (3/2015 Isolate #3)	ZMC	0810482CF	RSV Detected
40	Respiratory Syncytial Virus Type A (2006 isolate)	ZMC	0810040ACF	RSV Detected
41	Respiratory Syncytial Virus Type A (2014 Isolate 341)	ZMC	0810290CF	RSV Detected
42	Respiratory Syncytial Virus Type A (2014 Isolate 342)	ZMC	0810291CF	RSV Detected
43	Respiratory Syncytial Virus Type A (2/2015 Isolate #2)	ZMC	0810474CF	RSV Detected
44	Respiratory Syncytial Virus Type A (2/2015 Isolate #3)	ZMC	0810475CF	RSV Detected
45	Respiratory Syncytial Virus Type A (4/2015 Isolate #1)	ZMC	0810481CF	RSV Detected
46	Respiratory Syncytial Virus Type A (2013 Isolate)	ZMC	0810299CF	RSV Detected
47	Human respiratory syncytial virus A (Long)	ATCC	VR-26	RSV Detected
48	Respiratory Syncytial Virus Type B (CH93(18)-18)	ZMC	0810040CF	RSV Detected
49	Respiratory Syncytial Virus Type B (12/2014 Isolate #1)	ZMC	0810450CF	RSV Detected
50	Respiratory Syncytial Virus Type B (11/2014 Isolate #2)	ZMC	0810451CF	RSV Detected
51	Respiratory Syncytial Virus Type B (3/2015 Isolate #1)	ZMC	0810479CF	RSV Detected
52	Respiratory Syncytial Virus Type B (3/2015 Isolate #2)	ZMC	0810480CF	RSV Detected
53	Human respiratory syncytial virus B (Strain: 9320)	ATCC	VR-955	RSV Detected
54	SARS-CoV-2 isolate Australia/VIC01	TWIST BIOSCIENCE	102019	SARS-CoV-2 Detected
55	SARS-CoV-2 isolate Wuhan-Hu-1	TWIST BIOSCIENCE	102024	SARS-CoV-2 Detected
56	SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated	BEI	NR-52287	SARS-CoV-2 Detected
57	Human adenovirus 1	ATCC	VR-1	Not Detected
58	Human adenovirus 18	ATCC	VR-1095	Not Detected
59	Human adenovirus 2	KBPV	VR-58	Not Detected

NO.	Organism	Source	Isolate No.	Result†
60	Human adenovirus 23	ATCC	VR-1101	Not Detected
61	Human adenovirus 3	ATCC	VR-3	Not Detected
62	Human adenovirus 4	ATCC	VR-1572	Not Detected
63	Human adenovirus 5	KBPV	VR-61	Not Detected
64	Human adenovirus 8	ATCC	VR-1368	Not Detected
65	Human coronavirus 229E	ATCC	VR-740	Not Detected
66	Human coronavirus NL63	ZMC	0810228CF	Not Detected
67	Human coronavirus HKU1	Korean isolated sample		Not Detected
68	Human coronavirus OC43	ATCC	VR-1558	Not Detected
69	Human metapneumovirus	KBPV	VR-86	Not Detected
70	Human coxsackievirus A24	ATCC	VR-583	Not Detected
71	Human coxsackievirus A9	KBPV	VR-11	Not Detected
72	Human coxsackievirus B1	KBPV	VR-13	Not Detected
73	Human coxsackievirus B2	KBPV	VR-14	Not Detected
74	Human coxsackievirus B3	KBPV	VR-15	Not Detected
75	Human coxsackievirus B4	KBPV	VR-16	Not Detected
76	Human coxsackievirus B5	KBPV	VR-17	Not Detected
77	Human coxsackievirus B6	KBPV	VR-18	Not Detected
78	Human echovirus 11	KBPV	VR-22	Not Detected
79	Human Echovirus 22 (Parechovirus)	KBPV	VR-23	Not Detected
80	Human echovirus 25	KBPV	VR-24	Not Detected
81	Human echovirus 30	KBPV	VR-25	Not Detected
82	Human echovirus 6	KBPV	VR-19	Not Detected
83	Human echovirus 7	KBPV	VR-20	Not Detected
84	Human echovirus 9	ATCC	VR-39	Not Detected
85	Human enterovirus 70	ATCC	VR-836	Not Detected
86	Human enterovirus 71	ATCC	VR-784	Not Detected
87	Human herpesvirus 1	ATCC	VR-260	Not Detected
88	Human herpesvirus 2	ATCC	VR-734	Not Detected
89	Human parainfluenza virus 1	ATCC	VR-1380	Not Detected
90	Human parainfluenza virus 2	ATCC	VR-92	Not Detected
91	Human parainfluenza virus 3	ATCC	VR-93	Not Detected
92	Human parainfluenza virus 4A	ATCC	VR-1378	Not Detected
93	Human parainfluenza virus 4B	ATCC	VR-1377	Not Detected
94	Human rhinovirus 14	ATCC	VR-284	Not Detected

NO.	Organism	Source	Isolate No.	Result†
95	Human rhinovirus 16	ATCC	VR-283	Not Detected
96	Human rhinovirus 8	ATCC	VR-488	Not Detected
97	Human Rhinovirus A90	ATCC	VR-1291	Not Detected
98	*Pseudomonas aeruginosa*	ATCC	9027	Not Detected
99	*Streptococcus pneumoniae*	KCTC	5764	Not Detected
100	*Proteus mirabilis*	KCTC	2566	Not Detected
101	*Candida albicans*	ATCC	10231	Not Detected
102	*Pseudomonas fluorescens*	KCTC	42821	Not Detected
103	*Staphylococcus aureus*	ATCC	33591	Not Detected
104	*Streptococcus pyogenes*	ATCC	19615	Not Detected
105	*Streptococcus mitis*	KCCM	42897	Not Detected
106	*Bordetella pertussis*	ATCC	BAA-589	Not Detected
107	*Staphylococcus epidermidis*	KCTC	13172	Not Detected
108	*Chlamydophila pneumoniae*	ATCC	53592	Not Detected
109	*Enterobacter aerogenes*	ATCC	13048	Not Detected
110	*Enterobacter cloacae*	ZMC	801830	Not Detected
111	*Haemophilus influenzae*	KCCM	42099	Not Detected
112	*Klebsiella pneumoniae*	ATCC	BAA-1706	Not Detected
113	*Legionella pneumophila*	KCTC	12009	Not Detected
114	*Mycoplasma pneumoniae*	ATCC	15293	Not Detected
115	SARS-coronavirus, Tor2	ZMC	NATSARS-ST	Not Detected
116	MERS-coronavirus, EMC/2012	ZMC	NATMERS-ST	Not Detected
117	*Mycobacterium tuberculosis*	ATCC	25177	Not Detected
118	*Streptococcus salivarius*	KCTC	5512	Not Detected
119	Alphacoronavirus 1 (feline infectious Peritonitis Virus), 79-1146	BEI	NR-49097	Not Detected
120	Porcine Respiratory Coronavirus, ISU-1	BEI	NR-48572	Not Detected

† Specificity tests were repeated 3 times.

※ ATCC: American Type Culture Collection
BEI: BEI Resources
KBPV: Korea Bank for Pathogenic Viruses
KCCM: Korean Culture Center of Microorganisms
KCTC: Korean Collection for Type Cultures
ZMC: ZeptoMetrix Corporation

2. Analytical Sensitivity

The analytical sensitivity (limit of detection) of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was determined using limiting dilutions of following virus cultures: 3 Influenza A strains, each 1 strain of Influenza B, RSV A, RSV B and the gamma-irradiated form of SARS-CoV-2. Each viral strain was serially diluted into negative sample matrix. Nucleic acids were extracted from each dilution using automated extraction system (Microlab NIMBUS IVD, Universal Cartridge kit) and analyzed with Allplex™ SARS-CoV-2 Assay.

Detection target	Source	Strain	Limit of Detection ($TCID_{50}$/mL)	Positive /Total	Positive rate (%)
S gene	BEI (Cat. NR-52287)	USA-WA1/2020	0.14	40/40	100
RdRP gene			0.14	40/40	100
N gene			0.14	40/40	100
SARS-CoV-2*			0.028	39/40	97.5
RSV A	Zeptometrix (Cat. 0810040ACF)	2006 Isolate	0.525	40/40	100
RSV B	Zeptometrix (Cat. 0810040CF)	CH93(18)-18	0.210	40/40	100
Flu B	Zeptometrix (Cat. 0810255CF)	Florida/04/06	0.126	40/40	100
Flu A H1N1	Zeptometrix (Cat. 0810036CF)	New Cal/20/99	4.57	40/40	100
Flu A H1N1pdm	Zeptometrix (Cat. 0810165CF)	California/07/09	0.417	40/40	100
Flu A H3N2	Zeptometrix (Cat. 0810240CF)	Victoria/361/11	0.0701	40/40	100

* SARS-CoV-2 is considered as "detected" when one or more of three target genes are positive.

3. Reproducibility

The reproducibility test was prepared including High Negative (0.1 X LoD), Low positive (1XLoD) and Moderate positive (3XLoD) samples. At each testing site, the kit was tested for five days, two runs per day by two different experimenters and triplicate of each target. The positive rates were observed for each target for reproducibility study: 100.0% for Moderate positive samples, ≥95% for Low positive samples. The reproducibility of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was evaluated between runs, sites and product lots. Positive rates for all concentrations and CV values met criteria of less than 10 (<10).

The results were satisfied with the Criteria set above, thus confirming the reproducible performances of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay.

4. Interfering substances

There were not effects on the results by adding the substances: non-specific detections or inhibitions on target amplification. Based on the results, 7 interfering substances had no effect on Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay results.

No.	Interfering Substances	Source	Test Concentration
1	Mucin (bovine submaxillary gland, type I-S)	Sigma-Aldrich (Cat.No.M3895)	60 µg/ml
2	Mupirocin (Antibiotic, nasal ointment)	Sigma-Aldrich (Cat.No.1448901)	6.6 mg/ml
3	Oxymetazoline (Afrin Nasal Spray)	Sigma-Aldrich (Cat.No.O2378)	15% (v/v)
4	Blood	Human	2% (v/v)
5	Tobramycin (Antibacterial, systemic)	Sigma-Aldrich (Cat.No.T4014)	4.0 µg/mL
6	Zanamivir (Anti-viral drug-Relenza)	Sigma-Aldrich (Cat.No.SML0492)	3.3 mg/mL
7	Oseltamivir (Anti-viral drug-Tamiflu)	Sigma-Aldrich (Cat.No.1479304)	25 mg/mL

5. Clinical performance

A total of 292 specimens were included in this clinical performance. The specimens consist of 8 nasopharyngeal aspirate samples, 122 nasopharyngeal swab samples, 7 bronchoalveolar lavage samples, 75 oropharyngeal (throat) swab samples, and 80 sputum samples. Clinical performance of the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay was evaluated through the comparison with other SARS-CoV-2 real-time PCR assay and respiratory virus real-time PCR assay which are CE-IVD approved before. The result has shown higher than 95% of agreement in clinical samples. Therefore, it is confirmed that the quality of Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay is valid. The performance is summarized in table.

SARS-CoV-2		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 / FluA/FluB/RSV Assay	Positive	152	4*	156
	Negative	4**	132	136
	Total	156	136	292

- ORA(Overall rates of agreement): 97.26% (95% CI: 94.67% to 98.81%)
- PPA(Positive Percent Agreement): 97.44% (95% CI: 93.57% to 99.30%)
- NPA(Negative Percent Agreement): 97.06% (95% CI: 92.64% to 99.19%)
- Kappa value: 0.95 (95% CI: 0.91 to 0.98)

* Sequencing and confirms true positive.

** The samples showed late Ct values (37~40), which are indicative of viral loads below LoD.

Influenza A virus (Flu A)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 / FluA/FluB/RSV Assay	Positive	27	0	27
	Negative	0	265	265
	Total	27	265	292

- ORA(Overall rates of agreement): 100.00% (95% CI: 98.74% to 100.00%)
- PPA(Positive Percent Agreement): 100.00% (95% CI: 87.23% to 100.00%)
- NPA(Negative Percent Agreement): 100.00% (95% CI: 98.62% to 100.00%)
- Kappa value: 1.00 (95% CI: 1.00 to 1.00)

Influenza B virus (Flu B)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 / FluA/FluB/RSV Assay	Positive	7	0	7
	Negative	0	285	285
	Total	7	285	292

- ORA(Overall rates of agreement): 100.00% (95% CI: 98.74% to 100.00%)
- PPA(Positive Percent Agreement): 100.00% (95% CI: 59.04% to 100.00%)
- NPA(Negative Percent Agreement): 100.00% (95% CI: 98.71% to 100.00%)
- Kappa value: 1.00 (95% CI: 1.00 to 1.00)

Respiratory syncytial virus (RSV)		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 / FluA/FluB/RSV Assay	Positive	14	2*	19
	Negative	0	276	276
	Total	14	278	292

- ORA(Overall rates of agreement): 99.32% (95% CI: 97.55% to 99.92%)
- PPA(Positive Percent Agreement): 100% (95% CI: 76.84% to 100.00%)
- NPA(Negative Percent Agreement): 99.28% (95% CI: 97.43% to 99.91%)
- Kappa value: 0.93 (95% CI: 0.83 to 1.00)

* Sequencing and confirms true positive.

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SYMBOLS

Key to symbols used in the manual and labels.

Symbol	Explanation
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
PREMIX	Enzyme mix
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests

ORDERING INFORMATION

Cat. No.	Product	Size
Allplex™ series		
RV10260Y	Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay	50 rxns
RV10259X	**Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay**	**100 rxns***
* For use with the Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS, and Seegene STARlet only		
Accessory product		
SG1701	Ribo_spin vRD (Viral RNA/DNA Extraction Kit)	50 preps
Automated extraction systems		
65415-02	Microlab NIMBUS IVD	EA
173000-075	Microlab STARlet IVD	EA
65415-03	Seegene NIMBUS	EA
67930-03	Seegene STARlet	EA
744300.4.UC384	STARMag 96 X 4 Universal Cartridge Kit	384 T / 1box
EX00013C	STARMag 96 X 4 Viral DNA/RNA 200 C Kit	384 T / 1box
SG71100	SEEPREP32	EA
EX00009P	STARMag 96 ProPrep (Plate Type)	96 T / 1box
EX00009T	STARMag 96 ProPrep (Tube Type)	96 T / 1box

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SARS-COV-2, INFLUENZA AND RSV 8-WELL REF 20081

PANEL OF ASSAYS

CONSISTING OF THE IVD COMPONENTS:
STEP 1 TUBES FOR SARS-COV-2, INFLUENZA AND RSV 8-WELL REF 20081S
STEP 2 PLATES FOR SARS-COV-2, INFLUENZA AND RSV 8-WELL REF 20081P

FOR THE HIGH-PLEX 24 SYSTEM

INSTRUCTIONS FOR USE

IVD ARTG IVD CE

IN THE USA:
FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) OR BY STATE AUTHORIZATION OF CLIA-CERTIFIED HIGH COMPLEXITY LABORATORIES ONLY.

IMPORTANT NOTICE:

The **SARS-CoV-2 a** and **SARS-CoV-2 b** assays are now validated and suitable for IVD use.

If either of the SARS-CoV-2 assays calls positive, the sample can be treated as positive.

These Instructions for Use (IFU) must be read in conjunction with the High-Plex 24 System IFU.

TABLE OF CONTENTS

1. WARNINGS AND LIMITATIONS

- **IMPORTANT**: IVD performance claims are only applicable when the instructions provided are followed.
- **IMPORTANT**: Do not use if product or packaging is compromised in any way.
- **IMPORTANT**: Do not use Step 1 or Step 2 panel components with different catalogue and/or version numbers.
- **IMPORTANT**: Do not use expired products.
- Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.
- This panel of assays must only be used with the High-Plex 24 System.
- Good laboratory practice is essential for the intended performance of this panel of assays. For further safety information, please consult the relevant Safety Data Sheets (SDS). **Note**: No AusDiagnostics reagents contain hazardous substances, as listed in CLP Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) classification. AusDiagnostics SDS's can be accessed online at http://www.ausdiagnostics.com/regulatory.html
- This panel of assays is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this panel of assays should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.

2. FURTHER INSTRUCTIONS REQUIRED

The Instructions for Use (IFU) for SARS-CoV-2, Influenza and RSV 8-well includes panel-specific information not provided in other IFUs.The document ID of the IFU for this product is provided on the outer box label next to the IFU symbol and in the footer of this document. The IFU for this product and other IFUs can be accessed online at http://www.ausdiagnostics.com. Additionally, a paper copy is available upon request by contacting customer service via phone, email, or mail (see **Section 12. Technical Enquiries**).

These Instructions for Use must be read in conjunction with:

- High-Plex 24 System IFU (Document 91501-r13).
- Reagent Cassette IFU (Document 40001-r02)

and if applicable, must also be read with:

- Synthetic Positive Controls IFU (Document ID 91001-r08; See **Section 9. Controls**)
- Swab Elution Tubes (buffer) IFU (Document ID 90200r05; See **Section 6.3**)

3. NAME AND INTENDED USE

The SARS-CoV-2, Influenza and RSV 8-well panel of assays is intended for *in vitro* diagnostic (IVD) use by suitably trained personnel in qualified laboratories using the High-Plex 24 System (REF 91501).

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the enrichment of targets and then amplification of targeted DNA and/or RNA. For the full description of the principle of the method, see the High-Plex 24 System IFU, **Section 5. Principle of Method**.

The SARS-CoV-2, Influenza and RSV 8-well panel of assays is intended as a semi-automated IVD test for the identification of pathogens in nucleic acid extracts from appropriate specimen types. For specimen and sample types that may be used, please see **Section 6. Specimen Requirements**.

The pathogens targeted in this panel are listed and described in **Section 4. Summary and Explanation of the Test.**

4. SUMMARY AND EXPLANATION OF THE TEST

SARS-COV-2, INFLUENZA AND RSV 8-WELL REF 20081 VER 08

ARTG Identifier: 235809

Assay	Target
Influenza A	Influenza virus A (includes H1, H3, H5 and H7)
Flu Typing	Influenza virus A serotypes pdH1N1 and H3 (including H3N2) are differentiated
Influenza B	Influenza virus B (Yamagata and Victoria lineages)
RSV	Respiratory Syncytial Virus (includes and differentiates types A and B)
SARS-CoV-2 a	Severe Acute Respiratory Syndrome Coronavirus 2 (ORF1 gene)
SARS-CoV-2 b	Severe Acute Respiratory Syndrome Coronavirus 2 (ORF8 gene)
Sample Adequacy	Human reference gene for sample adequacy control
SPIKE	Artificial sequence for assay control

This product complies with the regulatory requirements for IVD medical devices of the competent authorities in Australia (ARTG Identifier 235809) and the European Union (CE-marked).

These tests utilise a multiplex-tandem polymerase chain reaction (MT-PCR)[2] for the amplification of targeted DNA and/or RNA. For the full description of the principle of the method, see the High-Plex 24 System IFU, **Section 5. Principle of the Method**.

The SARS-CoV-2, Influenza and RSV 8-well panel is intended to detect the following viruses that have been associated with respiratory infections:

4.1 TARGET DESCRIPTIONS: VIRUSES

Influenza A

Influenza virus A is the most virulent influenza virus in humans, and is sub-typed depending on surface haemagglutinin (HA) and neuraminidase (NA). The most significant H1 subtype has been H1N1, causing the Spanish Flu in 1918[3]. A novel H1N1 strain emerged in 2009 (pdH1N1 or "swine flu") that contained viral segments from human, pig and avian influenza[4]. The most common H3 variant is H3N2, which had its first known outbreak in 1968 in Hong Kong[5]. H5 and H7 variants are hosted by birds with certain strains transmissible to humans (causing "avian influenza") where sporadic infections are a public health concern. H5N1 has a mortality rate of about 60% of all known cases, where most sources of infection were from infected birds or contaminated environments[6]. Several H7 subtype infections with high pathogenicity have been reported in recent years, however there have been limited reports of human-to-human transmission[6]. The **Flu Typing** assay is designed to detect pdH1N1 and H3.

IMPORTANT: If the Flu Typing assay calls positive and the Influenza A assay calls negative, it is safe to report the Flu Typing assay as correct.

Influenza B

Influenza virus B is less common than Influenza A but does cause outbreaks of influenza as immunity is overcome by viral mutations. The Influenza Virus B assay is designed to detect both Influenza B variants, Victoria and Yamagata[7].

Respiratory Syncytial Virus (RSV)
Respiratory syncytial virus is a leading cause of lower respiratory tract infections in young children and accounts for 50 – 90% of bronchiolitis hospital admissions[3]. Out of the two strains in circulation, A and B, RSV A is the predominating strain. The RSV assay is designed to detect both RSV A and B, and the MT Analysis software will distinguish between the two strains.
IMPORTANT: Discrimination between RSV A and B strains is for information purposes only.

SARS-CoV-2
Coronaviruses are a large family of RNA viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The recently discovered coronavirus SARS-CoV-2 causes coronavirus disease COVID-19 and was first observed in Wuhan, China.
IMPORTANT: If either of the SARS-CoV-2 assays calls positive, the sample can be treated as positive.

5. PANEL COMPONENTS AND REAGENTS: MATERIALS AND STORAGE

Note: No AusDiagnostics reagents contain hazardous substances, as listed in Regulation (EC) No 1272/2008[1] and according to the Globally Harmonised System (GHS) classification.

Panel Name	Panel components	REF	GTIN
SARS-CoV-2, Influenza and RSV 8-well	Step 1 Tubes for SARS-CoV-2, Influenza and RSV 8-well	20081S	9343044001598
	Step 2 Plates for SARS-CoV-2, Influenza and RSV 8-well	20081S	9343044001581
To be used with:	Demi RNA Reagent Cassette	40341RNA	9343044003110

5.1 STEP 1 TUBES

Step 1 Tubes for SARS-CoV-2, Influenza and RSV 8-well panels contain tubes for 96 samples.

Materials	Label	Description	Function	Qty.
Step 1 Tubes	**STEP 1 TUBES**	1 x individual sealed bag containing 12 x 8-well tube strips with dried oligonucleotides.	Receptacle for the Step 1 reaction	96

STORAGE AND HANDLING INSTRUCTIONS

The Step 1 Tubes must be stored between 14°C - 29°C.

Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.2 STEP 2 PLATES

The Step 2 Plates box for SARS-CoV-2, Influenza and RSV 8-well panels contains materials for 288 samples.

Materials	Label	Description	Function	Qty.
Step 2 Plates box	**STEP 2 PLATES**	Outer box	Outer packaging	1
Step 2 Plate	**STEP 2 PLATE**	Sealed bag containing 384-well plate with dried oligonucleotides	Receptacle for the Step 2 PCR reaction	12
Dilution plate	D	Empty, 96-well plate	Houses the dilution for Step 2	12
(Bleach) Container	C	Empty, white-capped 5.0 mL tube	To be loaded with bleach, which deactivates DNA/ RNA	6
Tip disposal bag	T	Zip-lock bags	Collects used tips for safe disposal	6

STORAGE AND HANDLING INSTRUCTIONS

The Step 2 Plates box must be stored between 14°C - 29°C.

IMPORTANT: Ensure desiccant is intact before removing product from pouch; do not use product if desiccant is compromised or missing

Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.3 DEMI RNA REAGENT CASSETTE

Outer box label	Materials	Description
DEMI RNA REAGENT CASSETTE REF 40341RNA (2)	Step 1 RNA Enzymes	Enzymes for the Step 1 reaction including reverse transcriptase
	Step 1 Buffer	Buffer for the Step 1 reaction
	Step 2 Reagents	Enzymes in buffer for the Step 2 reaction
	Water	Used to dilute the Step 1 reaction products for Step 2
	Oil	Used to prevent evaporation of the Step 1 reaction

STORAGE AND HANDLING INSTRUCTIONS

Upon arrival, store the reagent cassettes below -20°C.

WARNING: Reagent cassettes may arrive thawed. This will not affect the performance of the reagent cassettes.

WARNING: A run must be started within 30 minutes of thawing the reagent cassette. Reagent cassettes are single-use only. Except for the initial freezing upon arrival (if necessary), do not re-freeze reagent cassettes.

Note: Take care to fully defrost reagent cassettes before **gently** inverting and spinning down as frozen reagents may pierce the foil seal.

Note: Do not re-use reagent cassettes. Dispose of them according to appropriate safety procedures.

Expiration of product is 6 months from manufacture and expiration date is provided on the label.

5.4 MATERIALS REQUIRED BUT NOT PROVIDED

Required reagents and equipment that are not provided by AusDiagnostics are:

- Personal protective equipment (PPE)
- Bleach with 0.4% available chlorine (4 mL required per run)
- Nuclease-free and adjustable pipettes
- Nuclease-free and sterile filtered tips

6. SPECIMEN REQUIREMENTS

6.1 SPECIMEN TYPES AND VOLUME

A nucleic acid extract that is suitable for PCR should be used with this product. Acceptable specimen types include nucleic acid extracts of nasal swabs, throat swabs, nasopharyngeal swabs, nasopharyngeal aspirate (NPA), tracheal aspirate, bronchial washing, or bronchoalveolar lavage (BAL).

Note: please store your nucleic acid extract in tubes free from PCR inhibitors and nucleases.

When working with RNA samples, standard precautions to minimise RNA degradation should be used.

IMPORTANT: Always handle and dispose of specimens potentially containing human pathogens according to relevant safety procedures.

Volume of sample to be added to Step 1 Tube must be 10 µL.

6.2 SUITABLE NUCLEIC ACID EXTRACTION METHODS

Manual extraction and pipetting of nucleic acid extracts into Step 1 Tubes is best performed in a biological safety cabinet or PCR setup area.

The following manual and automated nucleic acid extraction methods have been validated by AusDiagnostics customers, and have been deemed suitable to produce nucleic acid extracts compatible with the SARS-CoV-2, Influenza and RSV 8-well panel.

Extraction System	Type
AusDiagnostics MT-Prep Extractor	Automated
bioMerieux, NucliSENS easyMAG	Automated
PerkinElmer Chemagic Prepito-D	Automated
Qiagen EZ1 Advanced	Automated
Qiagen QIAamp	Manual
Qiagen QIAsymphony	Automated
Roche High Pure series	Manual
Roche MagNA Pure series	Automated

7. FURTHER MT ASSAY SET UP OPTIONS

This section is intended to be read in conjunction with the High-Plex 24 System IFU, **Section 7.3 Run the Processor**.

Additional MT Assay Setup software options are as follows:

Sampling: The table below explains the options provided for robotic sampling:

Sampling options	Applicable situation
Manual pipetting into tube strip	Nucleic acid extracts have been manually transferred to the Step 1 Tubes prior to starting the run (therefore no robotic sampling required).
Robot sampling from 2 mL tubes	Nucleic acid extracts stored in 2 mL tubes are loaded on the Autosampling block for robotic sampling. Minimum volume 40 µL
Robot sampling from 1.5 mL flip-cap tubes	Nucleic acid extracts are stored in 1.5 mL flip-cap tubes are loaded on the Autosampling block for robotic sampling. Minimum volume 40 µL
Robot sampling from non-sequential 96 well plate locations	Nucleic acid extracts are stored in a 96-well plate are placed in the Autosampling block section for robotic sampling of non-sequential wells on the plate. Minimum volume 40 µL
Robot sampling from 96 well plate samples 1-24	Nucleic acid extracts are stored in a 96-well plate are placed in the Autosampling block section for robotic sampling of wells 1 - 24. Minimum volume 40 µL
Robot sampling from 96 well plate samples 25-48	Nucleic acid extracts are stored in a 96-well plate are placed in the Autosampling block section for robotic sampling of wells 25 - 48. Minimum volume 40 µL
Robot sampling from 96 well plate samples 49-72	Nucleic acid extracts are stored in a 96-well plate are placed in the Autosampling block section for robotic sampling of wells 49 - 72. Minimum volume 40 µL
Robot sampling from 96 well plate samples 73-96	Nucleic acid extracts are stored in a 96-well plate are placed on the Autosampling block section for robotic sampling of wells 73 - 96. Minimum volume 40 µL

8. INTERPRETATION OF RESULTS

WARNING: IVD performance claims do not extend to any changes made by the user to a result (i.e. "Reject" or "Confirm" a result). Any user changes will be clearly indicated in the Analysis Report.

The cycling curves and the melt curves of a run are displayed in the MT Analysis Software. Based on predefined parameters, the software will call the target as 'Present', 'Check' or blank (not detected). Note that multiple infections are possible. Molecular target concentrations, expressed as arbitrary units, are calculated relative to the internal control SPIKE, which amplifies a known amount of target molecules. As the concentration of SPIKE is not measured these values are in arbitrary units. Furthermore, in some panels the relative concentration of each target may be inferred from the normalised percent (displayed in parentheses after the 'Present' call.

For further details on analysis of results, please refer to the High-Plex 24 System IFU (**Section 11. Interpreting Software Results**).

IMPORTANT: Please report results to state or local public health departments, if applicable.

9. CONTROLS

9.1 POSITIVE CONTROL

It is recommended that positive controls be included in every run. Please refer to individual laboratory procedures. The failure of a positive control should lead to the reassessment of any negative result obtained since the last control was run.

The Synthetic Positive Controls for Respiratory Pathogens (REF 91011 **VER 06**) contains all IVD targets for the SARS-CoV-2, Influenza and RSV 8-well panel except for SARS-CoV-2 b. A Synthetic Positive Control for the SARS-CoV-2 b assay is currently in development.

The Synthetic Positive Controls IFU must be read before using these controls.

9.2 NEGATIVE CONTROL

It is recommended that a negative control be run according to the individual laboratory procedures. Amplification of the negative control indicates contamination from the environment (e.g. from handling during set up or spillages of sample material on the MT Processor deck). In this case, the surface of the MT Processor deck (including the thermal cycler cover) should be wiped with a non-corrosive nucleic acid denaturing reagent (e.g. DNA-OFF™) and then UV treated. The relevant samples should be retested.
DO NOT USE BLEACH TO CLEAN THE INSTRUMENT.

9.3 SAMPLE ADEQUACY AND HUMAN DNA CONTROL

The Sample Adequacy Control assay targets a human reference gene as an indicator of the suitability of the nucleic acid extract, or direct sampling specimen. The Human DNA Control assay targets a human DNA reference gene, to indicate the presence of human DNA in the nucleic acid extract or direct sampling specimen.
IMPORTANT: If both the assay and sample adequacy control call negative, a new sample should be collected. If the assay calls positive and the sample adequacy calls negative, the sample is positive.

9.4 SUITABLE NUCLEIC ACID CONTROL

It is the user's responsibility to ensure a suitable nucleic acid extraction procedure is in place. It is recommended a known positive control be included per extraction run.

If the nucleic acid extraction control is not detected, the negative results cannot be relied upon. It is recommended that any sample with a negative nucleic acid extraction control should be re-collected and re-extracted if appropriate, and analysis repeated.

9.5 SAMPLE INHIBITION AND INSTRUMENT FUNCTION CONTROL

SPIKE is a completely artificial sequence that is present in Step 1 Tubes to monitor sample inhibition and instrument performance. SPIKE has been designed to have no cross-reactivity with diagnostic targets or assays. If SPIKE is shown to be inhibited, then this suggests that the sample contained inhibitory substances, or that the reaction conditions are suboptimal. In this case a negative result cannot be relied upon and it is recommended that the sample should be re-extracted if appropriate, and analysis repeated. For further details on analysis of SPIKE, please refer to the High-Plex 24 System IFU (**Section 9.2 Verification of Instrument Function and Sample Inhibition**).

10. PERFORMANCE CHARACTERISTICS

10.1 REPRODUCIBILITY AND REPEATABILITY

The reproducibility of the assays on the High-Plex 24 System was assessed by testing five samples on three batches across three days (with each batch tested once per day) and three systems with three operators. The coefficient of variation (c_V) for the resulting mean cycle take-off values (Ct values) for each of the samples were calculated. It was found that the Ct values for all samples at all concentrations were highly reproducible with c_V values ranging from 2.08% to 4.86%, averaging 3.40%.

The repeatability of the assays on the High-Plex 24 System was assessed by testing five samples on three batches with one batch tested three times each day on one system. It was found that the Ct values for all samples at all concentrations were highly repeatable with c_V values ranging from 2.26% to 5.11%, averaging 3.84%.

The low c_V from these studies provides evidence that the High-Plex 24 System is suitably precise for IVD use.

10.2 INTERFERING SUBSTANCES

A range of exogenous and endogenous substances including those expected to be found in the sample types for this panel were tested for potential PCR interference. Minimal or no interference was seen due to the presence of any one of the substances tested.

The presence of the internal control, SPIKE, in all AusDiagnostics panels controls for possible PCR interference in each sample.

For further details on interfering substances, please refer to the High-Plex 24 System IFU (**Section 13.1 Interfering Substances**).

10.3 ANALYTICAL SPECIFICITY

The MT-PCR technology achieves highly specific pathogen detection and is not to be confused with standard PCR or multiplex PCR. The step 1 amplification is limited to 18 cycles at which point very little of the starting materials (primers and dNTP) in the master mix have been converted into amplicon products. Each reaction therefore proceeds independently of the others in the multiplex thus preventing competition between the different assays. The second PCR step employs a set of individual PCR reactions with the primers nested inside the first step primers so that any mis-primed product in the first step cannot result in amplification in the second step. Furthermore the pre-amplification of the first step means that the second step PCR progresses easily to produce clean product in every case. Different conditions are employed for the two PCR steps which favour amplification of low concentration targets in the first step, but clean PCR in the second step. By using a two step reaction with a dilution between the steps, the effect of any inhibitory substances is also reduced.

A number of published studies have provided evidence that a positive result is only obtained for the gene targeted by the specific inner primers in the multiplex panels. For example, no cross-reactivity was detected in a 17 fungal pathogen multiplex using 200 blood culture specimens that contained bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, coagulase negative *Staphylococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Salmonella typhi*, *Bacteroides fragilis*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus gordonii*, *Streptococcus pyogenes*, *Streptococcus constellatus*, *Streptococcus salivarius*, *Sphingobacterium spiritivorum*, *Corynebacterium jeikeium*, *Propionibacterium acnes* or in 30 blood cultures without micro-organisms.[8, 9]

10.4 ANALYTICAL SENSITIVITY

The limit of detection (LoD) was determined using serial dilutions of plasmids, with a multiplexed Step 1 amplification and 16 replicates per dilution tested. The LoD was determined to be the lowest concentration that showed 100% target amplification. Ranges are used when samples with higher concentrations were not detected, contrary to 100% amplification of samples with lower concentrations. The LoDs calculation is given as either copies per 10 µL sample or copies per mL of the original sample. The sample copies/mL calculation is based on 100% efficient nucleic acid extraction that concentrates a 200 µL sample into a 50 µL eluate.

Assay	LoD (copies/ 10 µL)	LoD (copies/ mL)
Influenza A	76-95	1,900-2,375
H1(2009)	18	450
HA-H3	52	1300
Influenza B	21	525
RSV-A	4-27	100-675
RSV-B	85	2125
SARS-CoV-2 a	86-173	2150-4325
SARS-CoV-2 b	7	175

10.5 CLINICAL PERFORMANCE USING NUCLEIC ACID EXTRACTS

The clinical performance for the targets used in this product were assessed by multiple clinical laboratories in Australia, New Zealand and the United Kingdom. Each institution's alternative method was considered the reference method for this assessment.

Assay	SENSITIVITY % (95% CI)	SPECIFICITY % (95% CI)
Influenza A	100.0 (88.6-100.0)	100 (96.7-100.0)
H1(2009)	100.0 (80.8-100.0)	100.0 (97.0-100.0)
HA-H3	100.0 (82.8-100.0)	100.0 (97.1-100.0)
Influenza B	100.0 (87.4-100.0)	100.0 (99.2-100.0)
RSV (RSV A & B)	100.0 (93.9-100.0)	100.0 (99.1-100.0)
SARS-CoV-2 a	100 (90.8-100.0)	100 (97.4-100.0)
SARS-CoV-2 b	97.7 (86.5-99.9)	99.4 (96.2-100.0)

11. LIMITATIONS OF THE PROCEDURE

- This product is only for use by suitably trained personnel in qualified laboratories.
- The performance of the SARS-CoV-2, Influenza and RSV 8-well panel has only been established on the AusDiagnostics High-Plex 24 systems.
- The assays in this product do not provide a quantitative value for the pathogen(s) in the sample.
- The performance of the test has been evaluated for use with human specimen material only.
- The performance of this test has only been validated with nucleic acid extracts from the following specimen types: nasal swab, throat swab, nasopharyngeal swab, nasopharyngeal aspirate (NPA), tracheal aspiration, bronchial washing, and bronchoalveolar lavage (BAL). It has not been validated for use with other sample types.
- The performance of this test has not been established for patients without symptoms of respiratory illness.
- The performance of this test has not been established for immunocompromised individuals.
- The SARS-CoV-2, Influenza and RSV 8-well panel is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this product should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.
- The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (using manufacturer of specimen collection devices instructions). Failure to follow proper procedures can lead to incorrect results.
- Discrimination between RSV A and RSV B strains is for information purposes only.

12. TECHNICAL ENQUIRIES

Further instructions and troubleshooting can be found in the High-Plex 24 System IFU (**Section 14. Troubleshooting**).

For assistance or if any issues recur, please contact AusDiagnostics.
Email: support@ausdiagnostics.com

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13. ACKNOWLEDGEMENTS

[redacted]

14. GLOSSARY

The following symbols can be found on AusDiagnostics SARS-CoV-2, Influenza and RSV 8-well Panel components or throughout these Instruction for Use (IFU). Use the definitions below as a guideline to interpret the symbols.

14.1 SYMBOLS

Where relevant, symbols are taken from ISO 15223-1:2016 Medical devices - Symbols to be used with medical devices labels, labelling and information to be supplied.

	Manufacturer
	Authorised representative in the European Union
 or REF	Catalogue Number
 or Ver	Version number
LOT	Lot/Batch code
GTIN	This product has been assigned a unique Global Trade Item Number.
	Indicates that the relevant product is intended for *in vitro* diagnostic use in Australia and is included on the Australian Register of Therapeutic Goods (ARTG).
	Indicates that the relevant product is intended for *in vitro* diagnostic use in the European Economic Area and is compliant with the European IVD Directive 98/79/EC.
	WARNING. Please read the indicated section carefully.
	Please consult the identified instructions for use before use
	Do not re-use
	Storage temperature range (upper and lower limit)
	Storage temperature range (upper limit only)
	Positive Control
	Expiry date (yyyy-mm-dd)
WARNING	Indicates a statement that alerts users about a situations that, if not avoided, could result in hazards or other serious adverse consequences from the use of the device
IMPORTANT	Indicates a statement that alerts the user to special care or special activities necessary for the safe and effectives use of the device
Note	Indicates additional information

14.2 DEFINITIONS

Panel of assays: A set of panel components that are intended to be used together to detect a specific group ("panel") of targets. Panel components include Step 1 Tubes and Step 2 Plates.

Panel-specific IFU: Instructions for Use (IFU) which contain information specific to a panel.

Operator: The individual who is interacting with the High-Plex 24 System.

Primers: Short synthetic oligonucleotides specifically designed to bind to and amplify specific gene sequences under conditions provided during PCR.

A Run: All steps from starting the MT Assay Setup Software (see **Section 7.3 Run the processor**) to generation of the MT Analysis file is considered "a run"

RUO: RESEARCH USE ONLY. This assay is not intended for IVD use and has not been validated.

Target: A gene or sequence that primers are designed to hybridise to.

15. DISCLAIMER

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In no event shall AusDiagnostics be responsible for failures, errors, or other liabilities resulting from customers' noncompliance with the procedures and precautions outlined herein or as a result of pathogen variants which were not sequenced at the time of assay design.

16. REFERENCES

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17. DOCUMENT HISTORY

Document ID of IFU	Date of Change	Changes
20081-r01.1	17/04/2020	Added Limit of Detection data for SARS-CoV-2 b assay
20081-r01	20/03/2020	Original document.

35. Coronavirus SARS-COV-2 Testing

35.1 Test Mnemonic

RDL = DVIRAL
Test Codes = CORPCR

35.2 Purpose

This procedure describes an in-house molecular procedure for the detection of SAR-COV-2 by real-time PCR based on the method from the World Health Organisation (WHO) (*Diagnostic detection of 2019-nCoV by real-time RT-PCR version 2 - Protocol and preliminary evaluation as of Jan 17, 2020; Corman et.al., Charité Virology, Berlin, Germany*).

The method was adopted to meet the demand for SARS-COV-2 testing during the 2020 coronavirus crisis.

This method is a multiplexed one-step reverse transcription and PCR testing procedure targeting the E gene for detection of the SARS-COV-2 virus. A cellularity control targeting the Ribonuclease P protein subunit p30 (RPP30) gene is included to evaluate the adequacy of swab collection (Real-Time PCR for Measles Virus Detection on Clinical Specimens with Negative IgM Result in Morocco, Benamar et.al. PLOSONE | DOI:10.1371 / journal. pone.0147154, January 26, 2016).

Primer and probe sequences for SARS-COV-2 and RPP30 were checked in-silico from reference sequences in Genbank. The test is performed on the LightCycler 2.0 and LC480II.

35.3 Principle

The qualitative detection of SARS-COV-2 is achieved by amplification of the E gene.

The primer and probe sequences complement target regions in the SARS(1) and SARS-COV-2 viruses. This assay is therefore unable to differentiate between the two viruses.

Other coronaviruses are not detected by this method.

Pairwise Alignment

```
MH822886.1      ACGAATAGGGTTGTTCATAGTAAACTTTTTCAT--------------------------  27630
NC_019843.3     ACGAATAGGGTTGTTCATAGTAAACTTTTTCAT--------------------------  27642
MN908947.2      AGAGACAGGTACGTTAATAGTTAATAGCGTACT--------------------------  26313
AB257344.1      AGAAACAGGTACGTTAATAGTTAATAGCGTACT--------------------------  26128
NC_004718.3     AGAAACAGGTACGTTAATAGTTAATAGCGTACT--------------------------  26169
NC_006577.2     TGATACTGCTTGGTACATAGGACAGATTTTAGT--------------------------  27425
NC_006213.1     AGACACTGTGTGGTATGTGGGGCAAATAATTTT--------------------------  28166
NC_005831.2     GAAGAGCCTGTTGTTGGTATAGTCTACTCTTCT---------CAACTATACGAAGATGTT  25204
NC_002645.1     GAACACCCAGCTGTTGGAATAGTCAACACAGATTTCAAATTAGAAATCCACTAAGATGTT  24754
                    *       **                 *

MH822886.1      ------------------------------------TTTTACCGTAGTATGTGCTATAAC  27654
NC_019843.3     ------------------------------------TTTTACCGTAGTATGTGCTATAAC  27666
MN908947.2      ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT  26337
AB257344.1      ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT  26152
NC_004718.3     ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT  26193
NC_006577.2     ------------------------------------TTTAGTTTTATTTTGTCTTATTTC  27449
NC_006213.1     ------------------------------------TATAGTTGCCATTTGTTTATTGGT  28190
NC_005831.2     CCTTCGATTAATTGATGACAATGGTATTGTCCTCAATTCCATTTTATGGCTCCTTGTTAT  25264
NC_002645.1     CCTTAAGCTAGTGGATGATCATGCTTTGGTTGTTAATGTACTACTCTGGTGTGTGGTGCT  24814
                                                *                   *

MH822886.1      ACTCTTGGTGTGTATGGCTTTCCTTACGGCTACTAGATTATGTGTGCAATGTATGACAGG  27714
NC_019843.3     ACTCTTGGTGTGTATGGCTTTCCTTACGGCTACTAGATTATGTGTGCAATGTATGACAGG  27726
MN908947.2      CTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT  26397
AB257344.1      CTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT  26212
NC_004718.3     CTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT  26253
NC_006577.2     TTTAATCTTTGTTGTTGCTTTTTTAGCAACTATTAAGCTTTGTATGCAACTTTGTGGTTT  27509
NC_006213.1     TACAATAGTTGTAGTGGCATTTTTGGCAACTTTTAAATTGTGTATTCAACTTTGCGGTAT  28250
NC_005831.2     GATATTTTTCTTTGTGTTGGCAATGACCTTTATTAAACTG---ATTCAATTGTGTTTTAC  25321
NC_002645.1     TATAGTGATACTACTAGTGTGTATTACAATAATTAAACTA---ATTAAGCTTTGTTTCAC  24871
                    *  *     *       *  *     *    *       *
```

Primer/Probes

```
E_Sarbeco_F1    ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2    ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1    FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ
```

Key

```
MH822886.1              MERS CoV England
NC_019843.3             MERS coronavirus
MN908947.2              Wuhan-Hu-1 CoV
AB257344.1              SARS Frankfurt
NC_004718.3             SARS coronavirus
NC_006577.2             Human coronavirus HKU1
NC_006213.1             Human coronavirus OC43 ATCC VR-759
NC_005831.2             Human Coronavirus NL63
NC_002645.1             Human coronavirus 229E
```

Figure 1: *Nucleotide sequence alignment primers and probe against SARS-CoV2 and other coronaviruses at the E gene. The primers and probe are designed specifically for SARS, SARS Frankfurt and SARS-COV2. Other coronavirus strains have not been falsely detected by the primer and probe combination in studies conducted by the author (Corman et.al. 2020).*

35.4 Patient Preparation

Nucleic acid is extracted on the Magna Pure 96 platform from upper respiratory swabs according to method MP5.11.

35.5 Requirements

Controls
Positive Control (Verified patient acquired virus)
Artificial Positive control (QC).
Negative Extraction Control
Negative (PCR water) Control

PCR Reagents
Qiagen QuantiTect Multiplex RT-PCR NR (No Rox) Kit (Cat# 204843 (200) or 20484 (1000)).

Primers & Probes

RdRP_SARSr-R1 Wuhan	CAA ATG TTA AAA ACA CTA TTA GCA TA
RdRP_SARSr-F2	GTG ARA TGG TCA TGT GTG GCG G
RdRP_SARSr-R1	CAR ATG TTA AAS ACA CTA TTA GCA TA
RdRP_SARSr-P2	/56-FAM/CAG GTG GAA CCT CAT CAG GAG ATG C/3IABkFQ/
RdRP_SARSr-P1	/56-FAM/CCA GGT GGW ACR TCA TCM GGT GAT GC/3IABkFQ/
E_Sarbeco_F1	ACA GGT ACG TTA ATA GTT AAT AGC GT
E_Sarbeco_R2	ATA TTG CAG CAG TAC GCA CAC A
E_Sarbeco_P1	/56-FAM/ACA CTA GCC ATC CTT ACT GCG CTT CG/3IABkFQ/
HURNASE-F	AGA TTT GGA CCT GCG AGC G
HURNASE-R	GAG CGG CTG TCT CCA CAA GT
HURNASE-P	/5HEX/TTC TGA CCT GAA GGC TCT GCG CG/3IABkFQ/

ARTIFICIAL POSITIVE CONTROL (APC)
ACAGGTACGTTAATAGTTAATAGCGTAGTGAAATGGTCATGTGTGGCGGTCGTGGTATTCTTGCTAGTTACACTAGCCAT
CCTTACTGCGCTTCGACCAGGTGGAACCTCATCAGGAGATGCTTGTGTGCGTACTGCTGCAATATTTATGCTAATAGTGT
TTTTAACATTTG

Oligonucleotide Primer and Probe Preparation - All primers and probes are diluted in TEpH8 at 100uM to generate a *Stock Solution*. *Working stocks* are generated by diluting the Stock Solution to the required concentration in TEpH8.

50 X E gene Primer/Probe Mix (for LC480II)

50X E gene [final]		Volume to 1000uL
E_Sarbeco_F1	20uM	200uL
E_Sarbeco_R2	20uM	200uL
E_Sarbeco_P1	10uM	100uL
HURNASE-F	20uM	200uL
HURNASE-R	20uM	200uL
HURNASE-P	10uM	100uL

Aliquot 50uL to 20 amber microcentrifuge tubes. Produce appropriate labels as shown below.

E gene Mix 50X LC480
Incl HURNASE (50uL)
Batch:13/03/20Store-20^{o}C
Exp:13/03/2021SingleUseOnly

10uM E Primer/Probe mix (for Lightcycler 2.0)

A MIX (E gene)	[final]	Volume to 1000uL
E_Sarbeco_F1	10uM	100uL
E_Sarbeco_R2	10uM	100uL
E_Sarbeco_P1	10uM	100uL
HURNASE-F	10uM	100uL
HURNASE-R	10uM	100uL
HURNASE-P	10uM	100uL
TE pH8		400uL

APC

Stock is diluted to 50uM. The working dilutions are made at a dilution of 10^{11} which gives a CT value in the E gene assay around 30 cycles.

35.6 Calibration

Dual Colour Hydrolysis Probe Colour Compensation Procedure

A colour compensation is performed on the LightCycler 2.0 for each new batch of reagents.

A new colour compensation profile is necessary to control for variation between spectral profiles of fluorophores from different manufacturers and production batches.

Three reaction capillaries are set up.

1. BLANK: containing PCR buffer without primers, probes and template.

2. FAM: containing positive template, all primers and the FAM-labelled Hydrolysis probe only. (Note: Unquenched FAM emitted light (530nm) will be released during the amplification process).

3. HEX: containing positive template, all primers and the HEX-labelled Hydrolysis probe only. (Unquenched HEX emitted light (560nm) will be released during amplification process).

In the Clean Room, set up the three reactions and aliquot into three capillaries in duplicate to calibrate both LightCycler instruments (LightCycler 2 and 3) as follows:

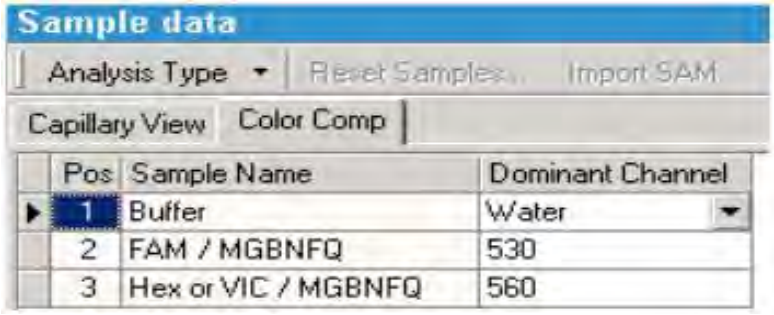
1. Label two LightCycler capillary tubes "1", two LightCycler capillary tubes "2" and two LightCycler capillary tubes "3".

2. Into the two tubes labelled "1", aliquot 20ul of Quantitect MasterMix and cap the tubes.

3. Prepare two 2.5x mastermixes at **10uM concentration** as follows: (note the water volume has been increased to make up to total volume 15ul mastermix).

Wuhan/SARS target (FAM) mastermix		Human IC (HEX) mastermix	
Quantitect MasterMix (Purple top)	25.0ul	Quantitect MasterMix (Purple top)	25.0ul
E_Sarbeco_F1	1.0ul	Human F primer	1.0ul
E_Sarbeco_R2	1.0ul	Human R primer	1.0ul
E_Sarbeco_ P1	1.0ul	Human probe	1.0ul
Quantitect enzyme (Red top)	0.5ul	Quantitect enzyme (Red top)	0.5ul
Water	9.0ul	Water	9.0ul
Total volume	37.5ul	Total volume	37.5ul

4. Mix gently, spin down and aliquot 15ul of the SARS CoV 2 (FAM) mastermix into each of the two capillary tubes labelled "2", and 15ul of the Human IC (HEX) mastermix into each of the two capillary tubes labelled "3".



5. Transfer the capillary tubes into the DNA laboratory. Place a set of tubes ('1", "2" and "3") into each of two LightCycler carousels.

6. Aliquot 5ul of RNA extract from a previously positive SARS CoV 2 patient into the four open capillaries (only tubes "2" and "3"). Cap the capillaries and transfer the carousels to the PCR products area.

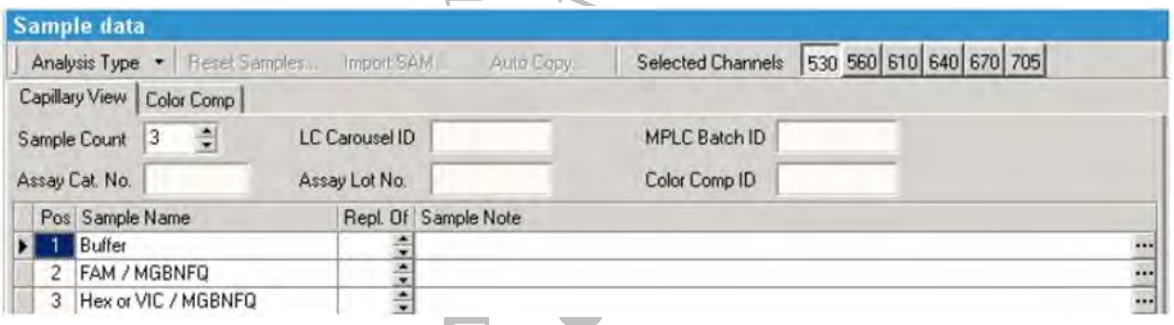
7. Place the carousels into the LightCycler centrifuge, spin and insert into the

LightCycler instruments.

1. The standard RT-PCR cycling conditions are performed BUT the colour compensation procedure must include an additional step called the *Temperature Gradient Step* (see parameters across).

2. Select Colour comp measles PCR from Run templates and set Max Seek Position to 3.

3. In the Sample Editor, enter the following information on the Capillary View tab:



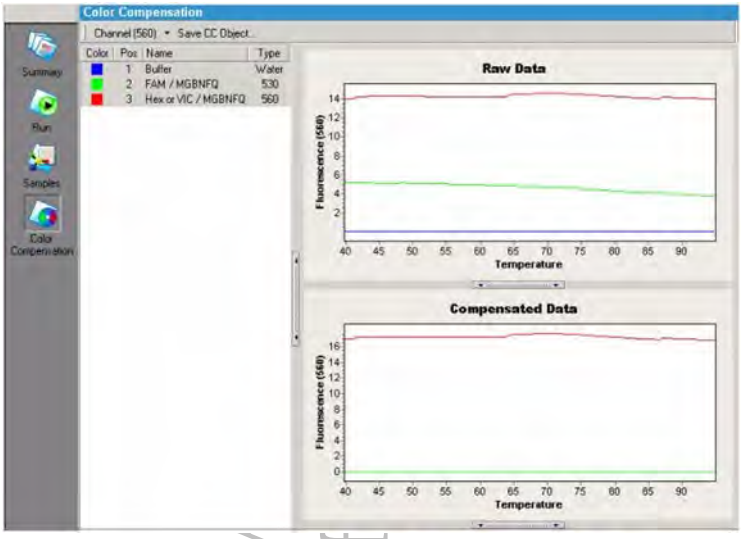
4. At the Colour Comp tab in Sample Editor, enter sample information as shown. Ensure that 530 & 560 are greyed out next to Selected Channels.

5. Load the capillaries on the carousel in order (ie. 1. BLANK, 2. FAM, 3.HEX).

6. Start the run. Save run as Colour Comp measles ddmmyy (***Run takes 2 hours).***

7. Once completed, select Colour Compensation from the Analysis menu.

8. Click Save “ccc object”.



9. Then navigate to the folder “Special Data\CCC” under the appropriate user name.

10. Save the file as a “ccc object” in that folder.

35.7 Calibrator Traceability

Not applicable to this assay.

35.8 Procedure

35.8.1 Specimen Check In

Specimens must have at least two identifiers (hospital patients must have three, patient name, date of birth and UR number) such as name, date of birth, UR number or address. For each specimen, check that the specimen details on the specimen and request form matches the records held in Kestral.

To check the above details in Kestral, perform the following steps:

1. Select Services in the Patient Data drop-down menu
2. In the Services Category Table Window, select Aliquots1.
3. In the Services Table for Category: Aliquots1, select Molec Aliquot. Press X to open. Scan the specimen barcode to open the patient data record screen. Check that the data entered matches the specimen.
4. Press ALT-D to open the request form image. Press ENTER at the View Images Window. If there is more than one, all images will need to be checked. Check that the test requested on the request from matches with the data entry in Kestral. The test code for Coronavirus PCR is: **CORPCR**.
5. Select the appropriate line (SWV/SWD/SWP/YT/WTNPA) and press SPACE BAR.
6. A new X number will be assigned and the specimen is then registered as received by the laboratory. Specimen labels are printed with the sample P & X number and other details (e.g. patient name, test requested).
7. Place an X number label on the primary specimen, on a 2ml screw top tube and also on a 1.5ml elution tube.

35.8.2 Printing Worksheets

Coronavirus PCR worksheets are printed using the Laboratory Information System (Kestral)

1. Log-in to Kestral using your assigned User Code and Password.
2. Select Services in the Patient Data drop-down men In the Services Category Table Window, select Molecular Worklists.
3. In the Services Table for Category: Molecular Worklists, select *Coronavirus worksheet*. Press X
4. Print out the worklist by selecting P.

   Select Q to quit the window. Press Escape (ESC) twice to return to the main menu. Collect the worksheet from the printer in the office

35.8.3 Preparation of the Master Mix

There are two methods based on the instrument – LC2.0 or LC480.

1. Prepare reagents for the master mix in a cold block in the cabinet in the PCR clean room according to Tables 1a and 1b as appropriate. Calculate the volumes of reagents required by multiplying volumes by the number of reactions required plus 10%.

2. After thawing, invert and centrifuge all reagents.

3. Prepare the mastermix in an amber 1.75mL tube.

4. In the cabinet, dispense the required volumes of mastermix into capillaries or a PCR plate for the LC2.0 or LC480II, respectively.

5. Add patient samples and all QC samples (SARS-COV-2 Positive extraction control, Artificial positive control, Extraction negative, PCR water negative) to respective capillaries or plate.

6. Close the capillaries or seal the plate with film. Centrifuge and load onto the LC2.0 or LC480 instrument as appropriate.

	A
	E gene + Human Control
2 X Quantitect MPLEX Master Mix (Purple)	10
A_E_Sarbeco_HURNA Mix (10uM)	0.4
Quantitect Mplex RT Mix (Red)	0.2
Water	4.4
Sample RNA	5
Total	20

Figure 1a: PCR Setup for LightCycler 2.0.

	A
	E gene + Human Control
2 X Quantitect MPLEX Master Mix (Purple)	12.5
A_E_Sarbeco_HURNASE Mix (50X)	0.5
Quantitect Mplex RT Mix (Red)	0.25
Water	1.75
Sample RNA	10
Total	25

\

Figure 1b: PCR Setup for LC480

LightCycler2.0 Coronavirus programme

The Coronavirus PCR cycling program is, Reverse Transcription (50^0C – 20mins [X1]; 95^0C – 15mins [X1]; 94^0C – 45sec, 60^0C – 45 sec, **(2-step cycling)** [X45] (Single Acquisition); Cooling 45^0C – 30 sec [X1].

Program the LightCycler as follows:

1) Reverse Transcription		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	50	
Hold	20mins	
Ramp rate (ºC/s)	20	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

2) PCR Initial activation step (Denaturation)		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	95	
Hold	15mins	
Ramp rate (ºC/s)	20	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

3) Amplification		
Cycles	45	
Analysis mode	Quantification	
Target temp ºC	94	60
Hold	45sec	45sec
Ramp rate (ºC/s)	20	20
Second target temp	0	0
Step size	0	0
Step Delay	0	0
Acquisition mode	None	Single

4) Cooling	
Cycles	1
Analysis Mode	None
Target Temp ºC	45
Hold	30sec
Ramp rate (ºC/s)	20
Second temp target	0
Step Size	0
Step Delay	0
Acquisition mode	None

LC480 Coronavirus programme

The Coronavirus PCR cycling program is, Reverse Transcription (50^0C – 20mins [X1]; 95^0C – 15mins [X1]; 94^0C – 45sec, 60^0C – 45 sec, **(2-step cycling)** [X45] (Single Acquisition); Cooling 45^0C – 30 sec [X1].

Program the LightCycler as follows:

5) Reverse Transcription		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	50	
Hold	20mins	
Ramp rate (ºC/s)	4.4	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

6) PCR Initial activation step (Denaturation)		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	95	
Hold	15mins	
Ramp rate (ºC/s)	4.4	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

7) Amplification		
Cycles	50	
Analysis mode	None	
Target temp ºC	94	60
Hold	45sec	45sec
Ramp rate (ºC/s)	2.2	2.2
Second target temp	0	0
Step size	0	0
Step Delay	0	0
Acquisition mode	None	Single

8) Cooling	
Cycles	1
Analysis Mode	None
Target Temp ºC	45
Hold	30sec
Ramp rate (ºC/s)	2.2
Second temp target	0
Step Size	0
Step Delay	0
Acquisition mode	None

35.8.4 Load and running the assay in the LightCycler 2.0

1. Place the carousel into the LightCycler 2.0 centrifuge and press start to spin down the reaction mix.

2. Whilst the carousel is spinning, go to the LightCycler and select the correct Run Protocol. Select Template from the top of the screen, Apply Template dialogue box opens. Select Run Templates, select Open.

3. Select the appropriate Protocol (Coronavirus 2020). Select Open.

4. To check that the correct Run Protocol has been selected, select Summary from the left hand side of the screen. The name of the Run Protocol will be printed at the bottom of the Summary, under Applied Templates.

5. In the Run screen, enter the number of samples to be tested (ie the number of capillary tubes in the carousel) in the Max Seek Pos box.

6. Select Samples from the left hand side of the screen. In the Samples screen, scan the sample identifier for all specimens on your run (X, N or P number, PCR negative, QC control). Return to the Run screen.

7. Remove the carousel from centrifuge & check reaction volumes are all the same. Place carousel into the LightCycler, ensuring that it is positioned correctly (position 1 at 1 O'clock) and sits flush with the surface

8. Close lid. Start the run by selecting Start Run. The Save LightCycler Experiment dialogue box appears.

9. Select Experiments, select the correct year, then select the correct test folder (eg Coronavirus 2020), and correct month folder (eg Coronavirus Feb 2020). Enter the experiment name (eg Coronavirus 06022020)) Select Save.

10. The program will start by checking that the correct number of capillaries can be detected, then start the cycling.

35.8.5 Load and running the assay in the LC480II

1. Switch ON the LC480II and computer.
2. At windows login enter [redacted]
3. Click on “LightCycler 480SW 1.5.1” to open the software.
4. At login enter [redacted]
5. To start a new experiment click on “New Experiment from Template”. Save the run in the format “DDMMYYYY HHMM COR”
6. Select “Coronavirus E gene RDRP Confirm”. Select Enter.
7. Load the plate containing the samples by pressing the button on the LC480 to open the holder. The new plate ID will appear in the software.
8. Select RUN.
9. Whilst the run is progressing, select “Sample Editor” and enter sample identification numbers according the worksheet.
10. Once the run has completed, select Analysis and “ABSQuant/2nd derivative method”.
11. Analyse the internal control in channel 533-580. Check that the Negative and APC are negative for amplification and all patient samples are positive for amplification.
12. Analyse the SARS-COV-2 test results in the 465-510 channel. Check the APC is positive for amplification with a CT between 29-34 cycles. Check the negative controls are negative for amplification. Analyse patient samples for amplification and record results and CT values on the worksheet. Interpret result as indicated in Table 1.

35.8.6 Result Analysis and Interpretation on the Lightcycler 2.0

At run completion, the Analysis tab at the top of the screen will become operable.

1. Analyse results by Qualitative Detection. Select Analysis. Then select "Create New Analysis". Select "Qualitative Detection" from under "Amplification Analysis".

2. In the Confirm dialogue box, select No. The results will be displayed.

3. Select the Channel drop down. Select the required channel (530 for E gene, FAM). Select the Channel drop down again and select None from the lower section (Select Channel Denominator). The channel is now set to 530/None.

4. From the top left-hand side, select the Colour Compensation (Off) drop down. Select the most current colour compensation object for the coronavirus assay.

5. Select the 530/560 colour compensation channels.

6. Enlarge the results screen by dragging the vertical line to the right. Select Advanced to show interpretation and the crossing points (ct) value.

7. Deselect positive calls and verify that all negative calls have no amplification curve.

8. Repeat this analysis for the internal control the by following steps 3-9 but selecting channels 560/None (HEX channel). Note Human RNase mRNA (the IC calls) crossing points in the HumRNA column of the worksheet.

9. Save the analysis by selecting Save from the top of the screen.

10. Print the results by selecting Report from the top of the screen. Note, the report cannot be printed until the analysis is saved.

11. From the right-hand side of the screen, select the experiment name and the Qualitative Detection files. The results will be displayed as they will be printed. Select the printer icon from the top of the screen, then click OK to print.

SARS CoV 2 Channel

Analyse results for SARS-COV-2 E gene virus using 530/None.

Record the Crossing Point value (C_p) for all positive amplification curves.

Internal Control Channel

Analyse results for the cellularity control using 560.

Pass/Fail Criteria

- The criteria for successful amplification of positive patient specimens are amplification beyond 0.05 fluorescence units at 530/None and 560/None.
- Any SARS-COV-2 not detected patient sample with no amplification in the Internal Control HEX 560 channel is INVALID. The test must be repeated for that sample. Repeated failure requires recollection.
- A positive patient may not have amplification in the HEX channel but only in the SARS-COV-2 target channel (530). This result is VALID.
- If the negative control indicates amplified product, the whole assay is INVALID and the assay repeated with a new negative control.

The below table summarises result scenarios and the associated interpretation.

E gene (FAM)	Internal Control (HEX)	INTERPRETATION
POSITIVE	NEGATIVE	VALID – SARS-COV-2 DETECTED. Repeat to confirm.
POSITIVE	POSITIVE	VALID – SARS-COV-2 DETECTED
NEGATIVE	POSITIVE	VALID – SARS-COV-2 NOT DETECTED
NEGATIVE	NEGATIVE	INVALID – RE-EXTRACT and REPEAT. Recollection may be necessary if test repeatedly fails.

35.8.7 Reporting Results - Laboratory Information System

Qualitative SARS-COV-2 testing results are reported as DETECTED or NOT DETECTED.

Results are entered in the Laboratory Information System (LIMS) and authorised by a second person as described below.

1. Log in to the Kestral LIS using your designated username and password.

2. At the main menu, select Patient Management and Services.

3. Select Molecular Worklist and then in the Services Table for Category: Molecular Worklists, select Viral and Bacterial Daybook

4. Select the appropriate sample using the arrow press Enter to open the record. Select E to edit the record and enter the data.

5. Press the down arrow key to reach the Specimen Type field. Press the F4 button to view the drop-down specimen options. Use the down arrow to move to the correct specimen type and hit enter to select.

6. Press the down arrow key three times to reach the RESULT field. Type d and hit F2 to populate the field with DETECTED or type ND and hit F2 to populate the field with Not Detected. The reporting comment is automatically applied.

35.8.8 Authorising Results

The results and data entry need to be checked by a second scientist before release.

35.8.9 Analytical Performance and Sensitivity

The analytical performance and sensitivity of the assays are described in the validation document.

35.9 Dilution/Concentrating Instructions

All primers and probes are diluted in TEpH8 to 100uM to generate a *Stock Solution*. *Working stocks* are generated by creating 50X working stocks. Refer to document MP10.02 for detailed instructions.

35.10 Internal Quality Control

An internal artificial positive control and a SARS-COV-2 Positive extraction control is included in every run.

A negative extraction control (water) is extracted with patient specimens and included in every run to ensure there is no contamination during the extraction stage or PCR set-up stage.

Internal QC results are recorded in LIMS.

35.11 EQAP

The laboratory is enrolled in a quality assurance program provided by Royal College of Pathologists QAP.

35.12 Method Limitations

This method detects SARS-COV-2 but cannot discriminate from SARS(1) coronavirus.

35.13 Clinically Significant Interferences

All positive cases are reviewed by a pathologist.

35.14 Potential Sources of Variability

Patient sample collection and storage conditions may affect assay performance.

35.15 Clinical Significance

This test was installed in response to the 2020 SARS-COV2 outbreak.

35.16 Action Results

For cases with low viral loads, a separate confirmatory assay or referral may be required.

35.17 Critical Results

Medical Officers are routinely notified of positive results and otherwise notifications occur on a case by case basis.

35.18 Kestral Alt-9 Information

Alt-9 information entered under test code “CoronaPCR”.

35.19 Safety Precautions

All samples must be handled carefully as though infectious.
Safe handling practices including Personal Protective Equipment (PPE) must be utilised when handling specimens, reagents and operating instrumentation.

35.20 Report Examples

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:
Requesting Dr: CLIN CHEM QC OFFICER

Report to:
TCH Act Pathology
Po Box 11
Woden 2606

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time: *UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **Not Detected**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **Not Detected**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing !!!!!UNAUTHORISED REPORT!!!!!
EUC,CORPCR
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/07/2020 13:08

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology

PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:

Requesting Dr: CLIN CHEM QC OFFICER

Report to:

TCH Act Pathology
Po Box 11
Woden 2606

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time:*UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **DETECTED**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **Not Detected**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, has been DETECTED.

Please refer to Public Health and Infection Prevention and Control advice regarding patient management and management of contacts.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing !!!!!UNAUTHORISED REPORT!!!!!
EUC,CORPCR
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/07/2020 13:08

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

Do

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology

PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:

Requesting Dr: CLIN CHEM QC OFFICER

Report to:

TCH Act Pathology
Po Box 11
Woden 2606

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 **SEX:** Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time: *UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2)	**Indeterminate**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**Not Detected**
Respiratory syncytial virus (RSV)	**Not Detected**

An INDETERMINATE result was obtained.

This may be due to low numbers of the novel coronavirus in the specimen or non-specific amplification of related viruses.

Interpretation of the results should be based on epidemiological risk and clinical features. Consideration should be made to repeat specimen collection. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing !!!!!UNAUTHORISED REPORT!!!!!
EUC,CORPCR
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/07/2020 13:09

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

Do

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology

PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:

Requesting Dr: CLIN CHEM QC OFFICER

Report to:

TCH Act Pathology
Po Box 11
Woden 2606

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time:*UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: 08/05/2020 Time:22:00

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **Not Detected**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **DETECTED**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing
EUC,CORPCR
AUTHORISED REPORT
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
Printed: 08/07/2020 13:06

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

Do

35.21 Associated Documentation

Nucleic Acid Extraction on Roche MagNA Pure Systems, MP05.11.
Oligonucleotide Primer and Probe Preparation, MP10.02.
QC & QA Specimens & Procedures, MP1.08.

35.22 References

Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39).

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76.

Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, et al. Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. J Virol. 2010;84(21):11336-49.

Muth D, Corman VM, Roth H, Binger T, Dijkman R, Gottula LT, et al. Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. Sci Rep. 2018;8(1):15177.

35. Coronavirus SARS-COV-2 Testing

35.1 Test Mnemonic

RDL = DVIRAL
Test Codes = CORPCR

35.2 Purpose

This procedure describes an in-house molecular procedure for the detection of SAR-COV-2 by real-time PCR based on the method from the World Health Organisation (WHO) (*Diagnostic detection of 2019-nCoV by real-time RT-PCR version 2 - Protocol and preliminary evaluation as of Jan 17, 2020; Corman et.al., Charité Virology, Berlin, Germany*).

The method was adopted to meet the demand for SARS-COV-2 testing during the 2020 coronavirus crisis.

This method is a multiplexed one-step reverse transcription and PCR testing procedure targeting the E gene for detection of the SARS-COV-2 virus. A cellularity control targeting the Ribonuclease P protein subunit p30 (RPP30) gene is included to evaluate the adequacy of swab collection (Real-Time PCR for Measles Virus Detection on Clinical Specimens with Negative IgM Result in Morocco, Benamar et.al. PLOSONE | DOI:10.1371 / journal. pone.0147154, January 26, 2016).

Primer and probe sequences for SARS-COV-2 and RPP30 were checked in-silico from reference sequences in Genbank. The test is performed on the LightCycler 2.0 and LC480II.

35.3 Principle

The qualitative detection of SARS-COV-2 is achieved by amplification of the E gene.

The primer and probe sequences complement target regions in the SARS(1) and SARS-COV-2 viruses. This assay is therefore unable to differentiate between the two viruses.

Other coronaviruses are not detected by this method.

Pairwise Alignment

```
MH822886.1       ACGAATAGGGTTGTTCATAGTAAACTTTTTCAT--------------------------          27630
NC_019843.3      ACGAATAGGGTTGTTCATAGTAAACTTTTTCAT--------------------------          27642
MN908947.2       AGAGACAGGTACGTTAATAGTTAATAGCGTACT--------------------------          26313
AB257344.1       AGAAACAGGTACGTTAATAGTTAATAGCGTACT--------------------------          26128
NC_004718.3      AGAAACAGGTACGTTAATAGTTAATAGCGTACT--------------------------          26169
NC_006577.2      TGATACTGCTTGGTACATAGGACAGATTTTAGT--------------------------          27425
NC_006213.1      AGACACTGTGTGGTATGTGGGGCAAATAATTTT--------------------------          28166
NC_005831.2      GAAGAGCCTGTTGTTGGTATAGTCTACTCTTCT---------CAACTATACGAAGATGTT          25204
NC_002645.1      GAACACCCAGCTGTTGGAATAGTCAACACAGATTTCAAATTAGAAATCCACTAAGATGTT          24754
                     *       **                  *

MH822886.1       ------------------------------------TTTTACCGTAGTATGTGCTATAAC          27654
NC_019843.3      ------------------------------------TTTTACCGTAGTATGTGCTATAAC          27666
MN908947.2       ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT          26337
AB257344.1       ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT          26152
NC_004718.3      ------------------------------------TCTTTTTCTTGCTTTCGTGGTATT          26193
NC_006577.2      ------------------------------------TTTAGTTTTATTTTGTCTTATTTC          27449
NC_006213.1      ------------------------------------TATAGTTGCCATTTGTTTATTGGT          28190
NC_005831.2      CCTTCGATTAATTGATGACAATGGTATTGTCCTCAATTCCATTTTATGGCTCCTTGTTAT          25264
NC_002645.1      CCTTAAGCTAGTGGATGATCATGCTTTGGTTGTTAATGTACTACTCTGGTGTGTGGTGCT          24814
                                                         *                   *

MH822886.1       ACTCTTGGTGTGTATGGCTTTCCTTACGGCTACTAGATTATGTGTGCAATGTATGACAGG          27714
NC_019843.3      ACTCTTGGTGTGTATGGCTTTCCTTACGGCTACTAGATTATGTGTGCAATGTATGACAGG          27726
MN908947.2       CTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT          26397
AB257344.1       CTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT          26212
NC_004718.3      CTTGCTAGTCACACTAGCCATCCTTACTGCGCTTCGATTGTGTGCGTACTGCTGCAATAT          26253
NC_006577.2      TTTAATCTTTGTTGTTGCTTTTTTAGCAACTATTAAGCTTTGTATGCAACTTTGTGGTTT          27509
NC_006213.1      TACAATAGTTGTAGTGGCATTTTTGGCAACTTTTAAATTGTGTATTCAACTTTGCGGTAT          28250
NC_005831.2      GATATTTTTCTTTGTGTTGGCAATGACCTTTATTAAACTG---ATTCAATTGTGTTTTAC          25321
NC_002645.1      TATAGTGATACTACTAGTGTGTATTACAATAATTAAACTA---ATTAAGCTTTGTTTCAC          24871
                     *  *     *       *  *     *    *       *
```

Primer/Probes

```
E_Sarbeco_F1   ACAGGTACGTTAATAGTTAATAGCGT
E_Sarbeco_R2   ATATTGCAGCAGTACGCACACA
E_Sarbeco_P1   FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ
```

Key

```
MH822886.1            MERS CoV England
NC_019843.3           MERS coronavirus
MN908947.2            Wuhan-Hu-1 CoV
AB257344.1            SARS Frankfurt
NC_004718.3           SARS coronavirus
NC_006577.2           Human coronavirus HKU1
NC_006213.1           Human coronavirus OC43 ATCC VR-759
NC_005831.2           Human Coronavirus NL63
NC_002645.1           Human coronavirus 229E
```

Figure 1: *Nucleotide sequence alignment primers and probe against SARS-CoV2 and other coronaviruses at the E gene. The primers and probe are designed specifically for SARS, SARS Frankfurt and SARS-COV2. Other coronavirus strains have not been falsely detected by the primer and probe combination in studies conducted by the author (Corman et.al. 2020).*

35.4 Patient Preparation

Nucleic acid is extracted on the Magna Pure 96 platform from upper respiratory swabs according to method MP5.11.

35.5 Requirements

Controls
Positive Control (Verified patient acquired virus)
Artificial Positive control (QC).
Negative Extraction Control
Negative (PCR water) Control

PCR Reagents
Qiagen QuantiTect Multiplex RT-PCR NR (No Rox) Kit (Cat# 204843 (200) or 20484 (1000)).

Primers & Probes

RdRP_SARSr-R1 Wuhan	CAA ATG TTA AAA ACA CTA TTA GCA TA
RdRP_SARSr-F2	GTG ARA TGG TCA TGT GTG GCG G
RdRP_SARSr-R1	CAR ATG TTA AAS ACA CTA TTA GCA TA
RdRP_SARSr-P2	/56-FAM/CAG GTG GAA CCT CAT CAG GAG ATG C/3IABkFQ/
RdRP_SARSr-P1	/56-FAM/CCA GGT GGW ACR TCA TCM GGT GAT GC/3IABkFQ/
E_Sarbeco_F1	ACA GGT ACG TTA ATA GTT AAT AGC GT
E_Sarbeco_R2	ATA TTG CAG CAG TAC GCA CAC A
E_Sarbeco_P1	/56-FAM/ACA CTA GCC ATC CTT ACT GCG CTT CG/3IABkFQ/
HURNASE-F	AGA TTT GGA CCT GCG AGC G
HURNASE-R	GAG CGG CTG TCT CCA CAA GT
HURNASE-P	/5HEX/TTC TGA CCT GAA GGC TCT GCG CG/3IABkFQ/

ARTIFICIAL POSITIVE CONTROL (APC)
ACAGGTACGTTAATAGTTAATAGCGTAGTGAAATGGTCATGTGTGGCGGTCGTGGTATTCTTGCTAGTTACACTAGCCAT
CCTTACTGCGCTTCGACCAGGTGGAACCTCATCAGGAGATGCTTGTGTGCGTACTGCTGCAATATTTATGCTAATAGTGT
TTTTAACATTTG

Oligonucleotide Primer and Probe Preparation - All primers and probes are diluted in TEpH8 at 100uM to generate a *Stock Solution*. *Working stocks* are generated by diluting the Stock Solution to the required concentration in TEpH8.

50 X E gene Primer/Probe Mix (for LC480II)

50X E gene [final]		Volume to 1000uL
E_Sarbeco_F1	20uM	200uL
E_Sarbeco_R2	20uM	200uL
E_Sarbeco_P1	10uM	100uL
HURNASE-F	20uM	200uL
HURNASE-R	20uM	200uL
HURNASE-P	10uM	100uL

Aliquot 50uL to 20 amber microcentrifuge tubes. Produce appropriate labels as shown below.

E gene Mix 50X LC480
Incl HURNASE (50uL)
Batch:13/03/20Store-20^{o}C
Exp:13/03/2021SingleUseOnly

10uM E Primer/Probe mix (for Lightcycler 2.0)

A MIX (E gene)	[final]	Volume to 1000uL
E_Sarbeco_F1	10uM	100uL
E_Sarbeco_R2	10uM	100uL
E_Sarbeco_P1	10uM	100uL
HURNASE-F	10uM	100uL
HURNASE-R	10uM	100uL
HURNASE-P	10uM	100uL
TE pH8		400uL

APC

Stock is diluted to 50uM. The working dilutions are made at a dilution of 10^{11} which gives a CT value in the E gene assay around 30 cycles.

35.6 Calibration

Dual Colour Hydrolysis Probe Colour Compensation Procedure

A colour compensation is performed on the LightCycler 2.0 for each new batch of reagents.

A new colour compensation profile is necessary to control for variation between spectral profiles of fluorophores from different manufacturers and production batches.

Three reaction capillaries are set up.

1. BLANK: containing PCR buffer without primers, probes and template.

2. FAM: containing positive template, all primers and the FAM-labelled Hydrolysis probe only. (Note: Unquenched FAM emitted light (530nm) will be released during the amplification process).

3. HEX: containing positive template, all primers and the HEX-labelled Hydrolysis probe only. (Unquenched HEX emitted light (560nm) will be released during amplification process).

In the Clean Room, set up the three reactions and aliquot into three capillaries in duplicate to calibrate both LightCycler instruments (LightCycler 2 and 3) as follows:

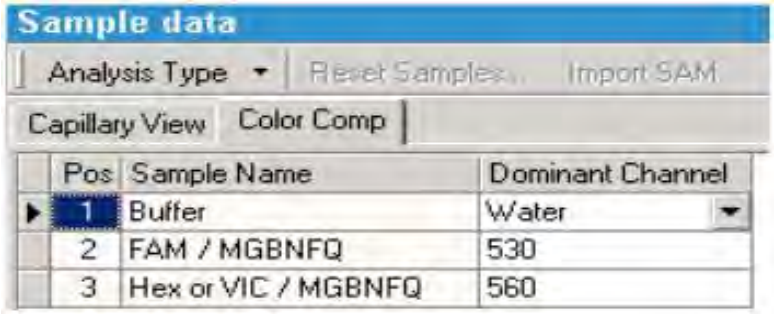
1. Label two LightCycler capillary tubes "1", two LightCycler capillary tubes "2" and two LightCycler capillary tubes "3".

2. Into the two tubes labelled "1", aliquot 20ul of Quantitect MasterMix and cap the tubes.

3. Prepare two 2.5x mastermixes at **10uM concentration** as follows: (note the water volume has been increased to make up to total volume 15ul mastermix).

Wuhan/SARS target (FAM) mastermix		Human IC (HEX) mastermix	
Quantitect MasterMix (Purple top)	25.0ul	Quantitect MasterMix (Purple top)	25.0ul
E_Sarbeco_F1	1.0ul	Human F primer	1.0ul
E_Sarbeco_R2	1.0ul	Human R primer	1.0ul
E_Sarbeco_ P1	1.0ul	Human probe	1.0ul
Quantitect enzyme (Red top)	0.5ul	Quantitect enzyme (Red top)	0.5ul
Water	9.0ul	Water	9.0ul
Total volume	37.5ul	Total volume	37.5ul

4. Mix gently, spin down and aliquot 15ul of the SARS CoV 2 (FAM) mastermix into each of the two capillary tubes labelled "2", and 15ul of the Human IC (HEX) mastermix into each of the two capillary tubes labelled "3".



5. Transfer the capillary tubes into the DNA laboratory. Place a set of tubes ('1", "2" and "3") into each of two LightCycler carousels.

6. Aliquot 5ul of RNA extract from a previously positive SARS CoV 2 patient into the four open capillaries (only tubes "2" and "3"). Cap the capillaries and transfer the carousels to the PCR products area.

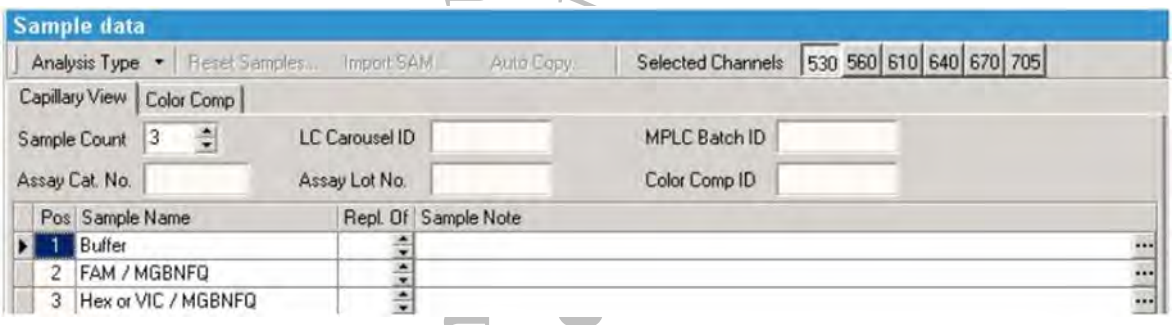
7. Place the carousels into the LightCycler centrifuge, spin and insert into the

LightCycler instruments.

1. The standard RT-PCR cycling conditions are performed BUT the colour compensation procedure must include an additional step called the *Temperature Gradient Step* (see parameters across).

2. Select Colour comp measles PCR from Run templates and set Max Seek Position to 3.

3. In the Sample Editor, enter the following information on the Capillary View tab:



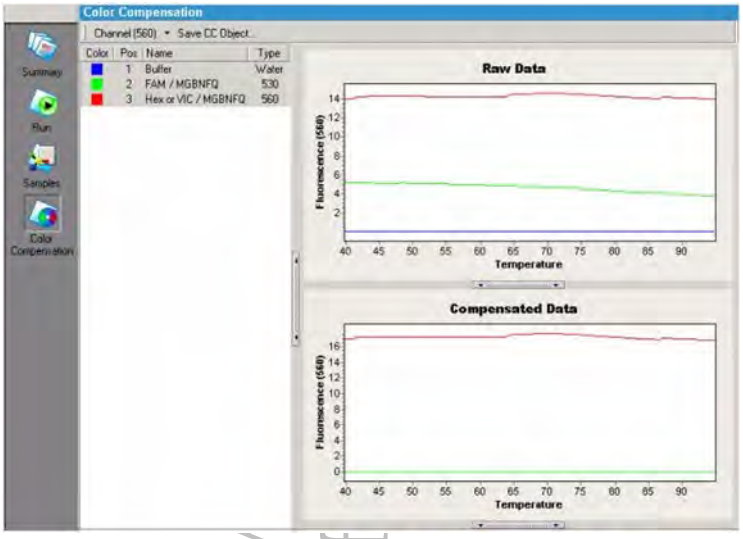
4. At the Colour Comp tab in Sample Editor, enter sample information as shown. Ensure that 530 & 560 are greyed out next to Selected Channels.

5. Load the capillaries on the carousel in order (ie. 1. BLANK, 2. FAM, 3.HEX).

6. Start the run. Save run as Colour Comp measles ddmmyy (***Run takes 2 hours).***

7. Once completed, select Colour Compensation from the Analysis menu.

8. Click Save “ccc object”.



9. Then navigate to the folder “Special Data\CCC” under the appropriate user name.

10. Save the file as a “ccc object” in that folder.

35.7 Calibrator Traceability

Not applicable to this assay.

35.8 Procedure

35.8.1 Specimen Check In

Specimens must have at least two identifiers (hospital patients must have three, patient name, date of birth and UR number) such as name, date of birth, UR number or address. For each specimen, check that the specimen details on the specimen and request form matches the records held in Kestral.

To check the above details in Kestral, perform the following steps:

1. Select Services in the Patient Data drop-down menu
2. In the Services Category Table Window, select Aliquots1.
3. In the Services Table for Category: Aliquots1, select Molec Aliquot. Press X to open. Scan the specimen barcode to open the patient data record screen. Check that the data entered matches the specimen.
4. Press ALT-D to open the request form image. Press ENTER at the View Images Window. If there is more than one, all images will need to be checked. Check that the test requested on the request from matches with the data entry in Kestral. The test code for Coronavirus PCR is: **CORPCR**.
5. Select the appropriate line (SWV/SWD/SWP/YT/WTNPA) and press SPACE BAR.
6. A new X number will be assigned and the specimen is then registered as received by the laboratory. Specimen labels are printed with the sample P & X number and other details (e.g. patient name, test requested).
7. Place an X number label on the primary specimen, on a 2ml screw top tube and also on a 1.5ml elution tube.

35.8.2 Printing Worksheets

Coronavirus PCR worksheets are printed using the Laboratory Information System (Kestral)

1. Log-in to Kestral using your assigned User Code and Password.
2. Select Services in the Patient Data drop-down men In the Services Category Table Window, select Molecular Worklists.
3. In the Services Table for Category: Molecular Worklists, select *Coronavirus worksheet*. Press X
4. Print out the worklist by selecting P.

   Select Q to quit the window. Press Escape (ESC) twice to return to the main menu. Collect the worksheet from the printer in the office

35.8.3 Preparation of the Master Mix

There are two methods based on the instrument – LC2.0 or LC480.

1. Prepare reagents for the master mix in a cold block in the cabinet in the PCR clean room according to Tables 1a and 1b as appropriate. Calculate the volumes of reagents required by multiplying volumes by the number of reactions required plus 10%.

2. After thawing, invert and centrifuge all reagents.

3. Prepare the mastermix in an amber 1.75mL tube.

4. In the cabinet, dispense the required volumes of mastermix into capillaries or a PCR plate for the LC2.0 or LC480II, respectively.

5. Add patient samples and all QC samples (SARS-COV-2 Positive extraction control, Artificial positive control, Extraction negative, PCR water negative) to respective capillaries or plate.

6. Close the capillaries or seal the plate with film. Centrifuge and load onto the LC2.0 or LC480 instrument as appropriate.

	A
	E gene + Human Control
2 X Quantitect MPLEX Master Mix (Purple)	10
A_E_Sarbeco_HURNA Mix (10uM)	0.4
Quantitect Mplex RT Mix (Red)	0.2
Water	4.4
Sample RNA	5
Total	20

Figure 1a: PCR Setup for LightCycler 2.0.

	A
	E gene + Human Control
2 X Quantitect MPLEX Master Mix (Purple)	12.5
A_E_Sarbeco_HURNASE Mix (50X)	0.5
Quantitect Mplex RT Mix (Red)	0.25
Water	1.75
Sample RNA	10
Total	25

\
Figure 1b: PCR Setup for LC480

LightCycler2.0 Coronavirus programme

The Coronavirus PCR cycling program is, Reverse Transcription (50^0C – 20mins [X1]; 95^0C – 15mins [X1]; 94^0C – 45sec, 60^0C – 45 sec, **(2-step cycling)** [X45] (Single Acquisition); Cooling 45^0C – 30 sec [X1].

Program the LightCycler as follows:

1) Reverse Transcription		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	50	
Hold	20mins	
Ramp rate (ºC/s)	20	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

2) PCR Initial activation step (Denaturation)		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	95	
Hold	15mins	
Ramp rate (ºC/s)	20	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

3) Amplification		
Cycles	45	
Analysis mode	Quantification	
Target temp ºC	94	60
Hold	45sec	45sec
Ramp rate (ºC/s)	20	20
Second target temp	0	0
Step size	0	0
Step Delay	0	0
Acquisition mode	None	Single

4) Cooling	
Cycles	1
Analysis Mode	None
Target Temp ºC	45
Hold	30sec
Ramp rate (ºC/s)	20
Second temp target	0
Step Size	0
Step Delay	0
Acquisition mode	None

LC480 Coronavirus programme

The Coronavirus PCR cycling program is, Reverse Transcription (50^0C – 20mins [X1]; 95^0C – 15mins [X1]; 94^0C – 45sec, 60^0C – 45 sec, **(2-step cycling)** [X45] (Single Acquisition); Cooling 45^0C – 30 sec [X1].

Program the LightCycler as follows:

5) Reverse Transcription		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	50	
Hold	20mins	
Ramp rate (ºC/s)	4.4	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

6) PCR Initial activation step (Denaturation)		
Cycles	1	
Analysis Mode	None	
Target Temp ºC	95	
Hold	15mins	
Ramp rate (ºC/s)	4.4	
Second temp target	0	
Step Size	0	
Step Delay	0	
Acquisition mode	None	

7) Amplification		
Cycles	50	
Analysis mode	None	
Target temp ºC	94	60
Hold	45sec	45sec
Ramp rate (ºC/s)	2.2	2.2
Second target temp	0	0
Step size	0	0
Step Delay	0	0
Acquisition mode	None	Single

8) Cooling	
Cycles	1
Analysis Mode	None
Target Temp ºC	45
Hold	30sec
Ramp rate (ºC/s)	2.2
Second temp target	0
Step Size	0
Step Delay	0
Acquisition mode	None

35.8.4 Load and running the assay in the LightCycler 2.0

1. Place the carousel into the LightCycler 2.0 centrifuge and press start to spin down the reaction mix.

2. Whilst the carousel is spinning, go to the LightCycler and select the correct Run Protocol. Select Template from the top of the screen, Apply Template dialogue box opens. Select Run Templates, select Open.

3. Select the appropriate Protocol (Coronavirus 2020). Select Open.

4. To check that the correct Run Protocol has been selected, select Summary from the left hand side of the screen. The name of the Run Protocol will be printed at the bottom of the Summary, under Applied Templates.

5. In the Run screen, enter the number of samples to be tested (ie the number of capillary tubes in the carousel) in the Max Seek Pos box.

6. Select Samples from the left hand side of the screen. In the Samples screen, scan the sample identifier for all specimens on your run (X, N or P number, PCR negative, QC control). Return to the Run screen.

7. Remove the carousel from centrifuge & check reaction volumes are all the same. Place carousel into the LightCycler, ensuring that it is positioned correctly (position 1 at 1 O'clock) and sits flush with the surface

8. Close lid. Start the run by selecting Start Run. The Save LightCycler Experiment dialogue box appears.

9. Select Experiments, select the correct year, then select the correct test folder (eg Coronavirus 2020), and correct month folder (eg Coronavirus Feb 2020). Enter the experiment name (eg Coronavirus 06022020)) Select Save.

10. The program will start by checking that the correct number of capillaries can be detected, then start the cycling.

35.8.5 Load and running the assay in the LC480II

1. Switch ON the LC480II and computer.
2. At windows login enter [redacted].
3. Click on “LightCycler 480SW 1.5.1” to open the software.
4. At login enter [redacted]
5. To start a new experiment click on “New Experiment from Template”. Save the run in the format “DDMMYYYY HHMM COR”
6. Select “Coronavirus E gene RDRP Confirm”. Select Enter.
7. Load the plate containing the samples by pressing the button on the LC480 to open the holder. The new plate ID will appear in the software.
8. Select RUN.
9. Whilst the run is progressing, select “Sample Editor” and enter sample identification numbers according the worksheet.
10. Once the run has completed, select Analysis and “ABSQuant/2nd derivative method”.
11. Analyse the internal control in channel 533-580. Check that the Negative and APC are negative for amplification and all patient samples are positive for amplification.
12. Analyse the SARS-COV-2 test results in the 465-510 channel. Check the APC is positive for amplification with a CT between 29-34 cycles. Check the negative controls are negative for amplification. Analyse patient samples for amplification and record results and CT values on the worksheet. Interpret result as indicated in Table 1.

35.8.6 Result Analysis and Interpretation on the Lightcycler 2.0

At run completion, the Analysis tab at the top of the screen will become operable.

1. Analyse results by Qualitative Detection. Select Analysis. Then select "Create New Analysis". Select "Qualitative Detection" from under "Amplification Analysis".

2. In the Confirm dialogue box, select No. The results will be displayed.

3. Select the Channel drop down. Select the required channel (530 for E gene, FAM). Select the Channel drop down again and select None from the lower section (Select Channel Denominator). The channel is now set to 530/None.

4. From the top left-hand side, select the Colour Compensation (Off) drop down. Select the most current colour compensation object for the coronavirus assay.

5. Select the 530/560 colour compensation channels.

6. Enlarge the results screen by dragging the vertical line to the right. Select Advanced to show interpretation and the crossing points (ct) value.

7. Deselect positive calls and verify that all negative calls have no amplification curve.

8. Repeat this analysis for the internal control the by following steps 3-9 but selecting channels 560/None (HEX channel). Note Human RNase mRNA (the IC calls) crossing points in the HumRNA column of the worksheet.

9. Save the analysis by selecting Save from the top of the screen.

10. Print the results by selecting Report from the top of the screen. Note, the report cannot be printed until the analysis is saved.

11. From the right-hand side of the screen, select the experiment name and the Qualitative Detection files. The results will be displayed as they will be printed. Select the printer icon from the top of the screen, then click OK to print.

SARS CoV 2 Channel

Analyse results for SARS-COV-2 E gene virus using 530/None.

Record the Crossing Point value (C_p) for all positive amplification curves.

Internal Control Channel

Analyse results for the cellularity control using 560.

Pass/Fail Criteria

- The criteria for successful amplification of positive patient specimens are amplification beyond 0.05 fluorescence units at 530/None and 560/None.
- Any SARS-COV-2 not detected patient sample with no amplification in the Internal Control HEX 560 channel is INVALID. The test must be repeated for that sample. Repeated failure requires recollection.
- A positive patient may not have amplification in the HEX channel but only in the SARS-COV-2 target channel (530). This result is VALID.
- If the negative control indicates amplified product, the whole assay is INVALID and the assay repeated with a new negative control.

The below table summarises result scenarios and the associated interpretation.

E gene (FAM)	Internal Control (HEX)	INTERPRETATION
POSITIVE	NEGATIVE	VALID – SARS-COV-2 DETECTED. Repeat to confirm.
POSITIVE	POSITIVE	VALID – SARS-COV-2 DETECTED
NEGATIVE	POSITIVE	VALID – SARS-COV-2 NOT DETECTED
NEGATIVE	NEGATIVE	INVALID – RE-EXTRACT and REPEAT. Recollection may be necessary if test repeatedly fails.

35.8.7 Reporting Results - Laboratory Information System

Qualitative SARS-COV-2 testing results are reported as DETECTED or NOT DETECTED.

Results are entered in the Laboratory Information System (LIMS) and authorised by a second person as described below.

1. Log in to the Kestral LIS using your designated username and password.

2. At the main menu, select Patient Management and Services.

3. Select Molecular Worklist and then in the Services Table for Category: Molecular Worklists, select Viral and Bacterial Daybook

4. Select the appropriate sample using the arrow press Enter to open the record. Select E to edit the record and enter the data.

5. Press the down arrow key to reach the Specimen Type field. Press the F4 button to view the drop-down specimen options. Use the down arrow to move to the correct specimen type and hit enter to select.

6. Press the down arrow key three times to reach the RESULT field. Type d and hit F2 to populate the field with DETECTED or type ND and hit F2 to populate the field with Not Detected. The reporting comment is automatically applied.

35.8.8 Authorising Results

The results and data entry need to be checked by a second scientist before release.

35.8.9 Analytical Performance and Sensitivity

The analytical performance and sensitivity of the assays are described in the validation document.

35.9 Dilution/Concentrating Instructions

All primers and probes are diluted in TEpH8 to 100uM to generate a *Stock Solution*. *Working stocks* are generated by creating 50X working stocks. Refer to document MP10.02 for detailed instructions.

35.10 Internal Quality Control

An internal artificial positive control and a SARS-COV-2 Positive extraction control is included in every run.

A negative extraction control (water) is extracted with patient specimens and included in every run to ensure there is no contamination during the extraction stage or PCR set-up stage.

Internal QC results are recorded in LIMS.

35.11 EQAP

The laboratory is enrolled in a quality assurance program provided by Royal College of Pathologists QAP.

35.12 Method Limitations

This method detects SARS-COV-2 but cannot discriminate from SARS(1) coronavirus.

35.13 Clinically Significant Interferences

All positive cases are reviewed by a pathologist.

35.14 Potential Sources of Variability

Patient sample collection and storage conditions may affect assay performance.

35.15 Clinical Significance

This test was installed in response to the 2020 SARS-COV2 outbreak.

35.16 Action Results

For cases with low viral loads, a separate confirmatory assay or referral may be required.

35.17 Critical Results

Medical Officers are routinely notified of positive results and otherwise notifications occur on a case by case basis.

35.18 Kestral Alt-9 Information

Alt-9 information entered under test code “CoronaPCR”.

35.19 Safety Precautions

All samples must be handled carefully as though infectious.
Safe handling practices including Personal Protective Equipment (PPE) must be utilised when handling specimens, reagents and operating instrumentation.

35.20 Report Examples

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:
Requesting Dr: CLIN CHEM QC OFFICER

Report to:
TCH Act Pathology
Po Box 11
Woden 2606

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time: *UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **Not Detected**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **Not Detected**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing !!!!!UNAUTHORISED REPORT!!!!!
EUC,CORPCR
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/07/2020 13:08

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology

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GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time:*UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **DETECTED**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **Not Detected**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, has been DETECTED.

Please refer to Public Health and Infection Prevention and Control advice regarding patient management and management of contacts.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

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SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 **SEX:** Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time: *UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2)	**Indeterminate**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**Not Detected**
Respiratory syncytial virus (RSV)	**Not Detected**

An INDETERMINATE result was obtained.

This may be due to low numbers of the novel coronavirus in the specimen or non-specific amplification of related viruses.

Interpretation of the results should be based on epidemiological risk and clinical features. Consideration should be made to repeat specimen collection. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

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Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/07/2020 13:09

Accredited for compliance with NPAAC Standards and ISO 15189.
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ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 10/01/2020 SEX: Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time:*UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: 08/05/2020 Time:22:00

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SARS-CoV-2).....: **Not Detected**
Influenza A.....: **Not Detected**
Influenza A Typing.....: **Not Detected**
Influenza B.....: **DETECTED**
Respiratory syncytial virus (RSV)...: **Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing
EUC,CORPCR
AUTHORISED REPORT
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
Printed: 08/07/2020 13:06

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
Page 1 of 1

Do

35.21 Associated Documentation

Nucleic Acid Extraction on Roche MagNA Pure Systems, MP05.11.
Oligonucleotide Primer and Probe Preparation, MP10.02.
QC & QA Specimens & Procedures, MP1.08.

35.22 References

Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39).

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1967-76.

Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, et al. Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. J Virol. 2010;84(21):11336-49.

Muth D, Corman VM, Roth H, Binger T, Dijkman R, Gottula LT, et al. Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. Sci Rep. 2018;8(1):15177.

39. Panther Aptima SARS-COV-2

39.1 Test Mnemonic

LIMS Code = CORPCR

39.2 Purpose

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19.

This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.2 These droplets also can land on objects and surfaces around the person. Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth. The virus that causes COVID-19 is infecting people and spreading easily from person to person.

On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).

The Aptima™ SARS-CoV-2 assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasopharyngeal (NP), nasal, mid-turbinate and oropharyngeal (OP) swab specimens, nasopharyngeal wash/ aspirate or nasal aspirates obtained from individuals meeting COVID-19 clinical and/or epidemiological criteria.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.

Positive results do not rule out bacterial infection or co-infection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Aptima SARS-CoV-2 assay on the Panther™ system is intended for use by clinical laboratory personnel specifically instructed and trained in the operation of the Panther and Panther Fusion systems and in vitro diagnostic procedures.

39.3 Principle

The Aptima SARS-CoV-2 assay combines the technologies of target capture, Transcription Mediated Amplification (TMA), and Dual Kinetic Assay (DKA).

Specimens are collected and transferred into their respective specimen transport tubes. The transport solutions in these tubes release the RNA target and protect them from degradation during storage. When the Aptima SARS-CoV-2 assay is performed in the laboratory, the target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the target molecules as well as a string of deoxyadenosine residues. A separate capture oligomer is used for each target. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the target molecules. The capture oligomer:target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured target molecules bound to them, are pulled to the side of the reaction vessel using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the specimens are ready for amplification.

Target amplification assays are based on the ability of complementary oligonucleotide primers to specifically anneal and allow enzymatic amplification of the target nucleic acid strands.

The Aptima SARS-CoV-2 assay replicates specific regions of the RNA from SARS-CoV-2 virus. Detection of the RNA amplification product sequences (amplicon) is achieved using nucleic acid hybridization. Single-stranded chemiluminescent nucleic acid probes, which are unique and complementary to a region of each target amplicon and Internal Control (IC) amplicon, are labelled with different acridinium ester (AE) molecules. The AE labelled probes combine with amplicon to form stable hybrids. The Selection Reagent differentiates hybridized from unhybridized probe, eliminating the generation of signal from unhybridized probe. During the detection step, light emitted from the labelled hybrids is measured as photon signals in a luminometer and are reported as Relative Light Units (RLU). In DKA, differences in the kinetic profiles of the labelled probes allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for the IC signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection reaction for the SARS-CoV-2 signal is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

The Aptima SARS-CoV-2 assay amplifies and detects two conserved regions of the ORF1ab gene in the same reaction, using the same “glower” kinetic type. The two regions are not differentiated and amplification of either or both regions lead to an RLU signal. The assay results are determined by a cut-off based on the total RLU and the kinetic curve type.

39.4 Patient Preparation

Specimen Collection and Storage

Specimens
Clinical material collected from patient placed in an appropriate transport system including in viral transport medium (VTM/UTM), saline, Liquid Amies, or specimen transport medium (STM). For the Aptima SARS-CoV-2 assay, suitable specimens include nasopharyngeal (NP), nasal, midturbinate and oropharyngeal (OP) swab specimens, bronchial washing, bronchoalveolar lavage, nasopharyngeal wash/aspirate and nasal aspirate specimen collections.

Swab Specimen Collection

Collect NP swab, nasal swab, and OP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3mL of VTM or UTM. Swab specimens may alternatively be added to saline, Liquid Amies or STM. The Aptima Multitest Swab Specimen Collection Kit may be used for the collection of OP and nasal swab samples.

After collection, specimens collected in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transferring to the Specimen Lysis Tube or transfer tubes as described in the specimen processing section below. Remaining specimen volumes can be stored at -70°C.

After collection, specimens in the Aptima Multitest Tube may be stored at 2°C to 30°C up to 6 days.

Note: Do not use medium that may contain Guanidium thiocyanate or any guanidine-containing material.

Specimen Processing using the Hologic Specimen Lysis Tube with Solid Cap

A. In the Biohazard Cabinet, uncap the pre-spun Hologic Specimen Lysis Tube and retain the cap. All Hologic Lysis tubes should be pre-spun to collect media to the bottom of the tube. Spin at 3000 rpm for 1 minute in the large Eppendorf Centrifuge.

B. Prior to testing on the Panther system and in a Biohazard Cabinet:
- Swab or aspirate: Transfer 500 µL of the specimen to a pre-spun Panther Fusion Specimen Lysis Tube, or
- Bronchial washing and bronchoalveolar lavage: Transfer 250 µL of the specimen (avoid transferring mucus) and 250 µL of STM to a pre-spun Panther Fusion Specimen Lysis Tube.

C. It is recommended to recap the tube and gently invert three times to ensure viral inactivation and a homogeneous mixture. Centrifuge the mixture for 1 minute at 3000 rpm in the large Eppendorf Centrifuge.

D. At the Panther instrument, place on appropriate PPE including face mask and shield along with new gloves. To avoid contact with the top of the tube, loosen the cap, and place the sample tube into the sample rack.

E. Remove and discard the cap. Inspect the sample tube. If bubbles are present, carefully remove from the sample tube using the tip of a sterile swab or similar method.

F. Place the rack retainer on the sample rack and load the rack into the instrument.
Note: Specimen processing using the Hologic Specimen Lysis Tube is for use with the Aptima SARS-CoV-2 uncapped tube assay software.

Sample Storage

1. Samples in the Aptima Multitest Tube, Aptima Specimen Tube, or Specimen Lysis Tube should be stored upright in the rack under the following condition: • 2°C to 30°C up to 6 days

2. The samples should be covered with a new and clean lid, plastic film or foil barrier.

Note: The Fisherbrand™ VersaClosure™ tube closure should not be used to cover tubes for freezing or shipping.

39.5 Requirements

Reagent Storage and Handling Requirements

A. The following reagents are stable when stored at 2°C to 8°C (refrigerated):

Aptima SARS-CoV-2 Amplification Reagent
Aptima SARS-CoV-2 Enzyme Reagent
Aptima SARS-CoV-2 Probe Reagent
Aptima SARS-CoV-2 Internal Control
Aptima SARS-CoV-2 Positive Control
Aptima SARS-CoV-2 Negative Control

B. The following reagents are stable when stored at 2°C to 30°C:

Aptima SARS-CoV-2 Amplification Reconstitution Solution
Aptima SARS-CoV-2 Enzyme Reconstitution Solution
Aptima SARS-CoV-2 Probe Reconstitution Solution
Aptima SARS-CoV-2 Selection Reagent

Note: Selection Reagent contains BORIC ACID 1-5% WARNING H315 - Causes skin irritation

C. The following reagents are stable when stored at 15°C to 30°C (room temperature):

Aptima SARS-CoV-2 Target Capture Reagent
Aptima Wash Solution
Aptima Buffer for Deactivation Fluid
Aptima Oil Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate. E. After reconstitution, the Enzyme Reagent, Amplification Reagent, and Probe Reagent are stable for 30 days when stored at 2°C to 8°C.

F. Discard any unused reconstituted reagents and wTCR after 30 days or after the Master Lot expiration date, whichever comes first.

G. Reagents stored on-board the Panther System have 72 hours of on-board stability.

H. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light. The specified reconstituted stability is based on 12 hours exposure of the Reconstituted Probe Reagent to two 60W fluorescent bulbs, at a distance of 17 inches (43 cm), and temperature less than 30°C. Light exposure of the Reconstituted Probe Reagent should be limited accordingly.

I. Upon warming to room temperature, some control tubes may appear cloudy or contain precipitates. Cloudiness or precipitation associated with controls does not affect control performance. The controls may be used whether they are clear or cloudy/precipitated. If clear controls are desired, solubilization may be expedited by incubating them at the upper end of the room temperature range (15°C to 30°C).

39.6 Calibration

Internal QC materials are run every 24 hours before testing.

A Preventative maintenance and calibration of the Panther instrument is conducted by the manufacturer at specified intervals.

Daily, Weekly and Monthly maintenance is conducted by laboratory staff.

39.7 Procedure

Panther System Test Procedure

Note: Quick Reference - See MP7.26 Panther System SASSCOV2 Assay Operator Checklist

Note: Refer to the Panther System Operator's Manual for additional procedural information.

A. Work Area Preparation

Clean work surfaces where reagents and samples will be prepared. Wipe down work surfaces with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and then follow with a water rinse. Do not allow

the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents and samples will be prepared with clean, plastic-backed absorbent laboratory bench covers. Repeat this procedure daily.

B. Reagent Reconstitution/Preparation of a New Kit
Note: Reagent reconstitution should be performed prior to beginning any work on the Panther System.

1. Reconstitute Amplification, Enzyme, and Probe Reagents

Combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.

a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and reagent have matching label colours before attaching the reconstitution collar.

b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.

c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).

d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.

e. While holding the reconstitution solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).

f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).

g. Thoroughly mix the solution in the glass vial by swirling (Figure 1, Step 4).

h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.

i. Remove the reconstitution collar and glass vial (Figure 1, Step 6).

j. Recap the plastic bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).

k. Discard the reconstitution collar and glass vial (Figure 1, Step 8).
Option: Additional mixing of the Amplification, Enzyme, and Probe Reagents using a tube rocker is allowed. The reagents may be mixed by placing the recapped plastic bottle on a tube rocker set to 20 RPM (or equivalent) for a minimum of 5 minutes.

Warning: Avoid creating foam when reconstituting reagents. Foam compromises the level sensing in the Panther System. Warning: Adequate mixing of the reagents is necessary to achieve expected assay results.

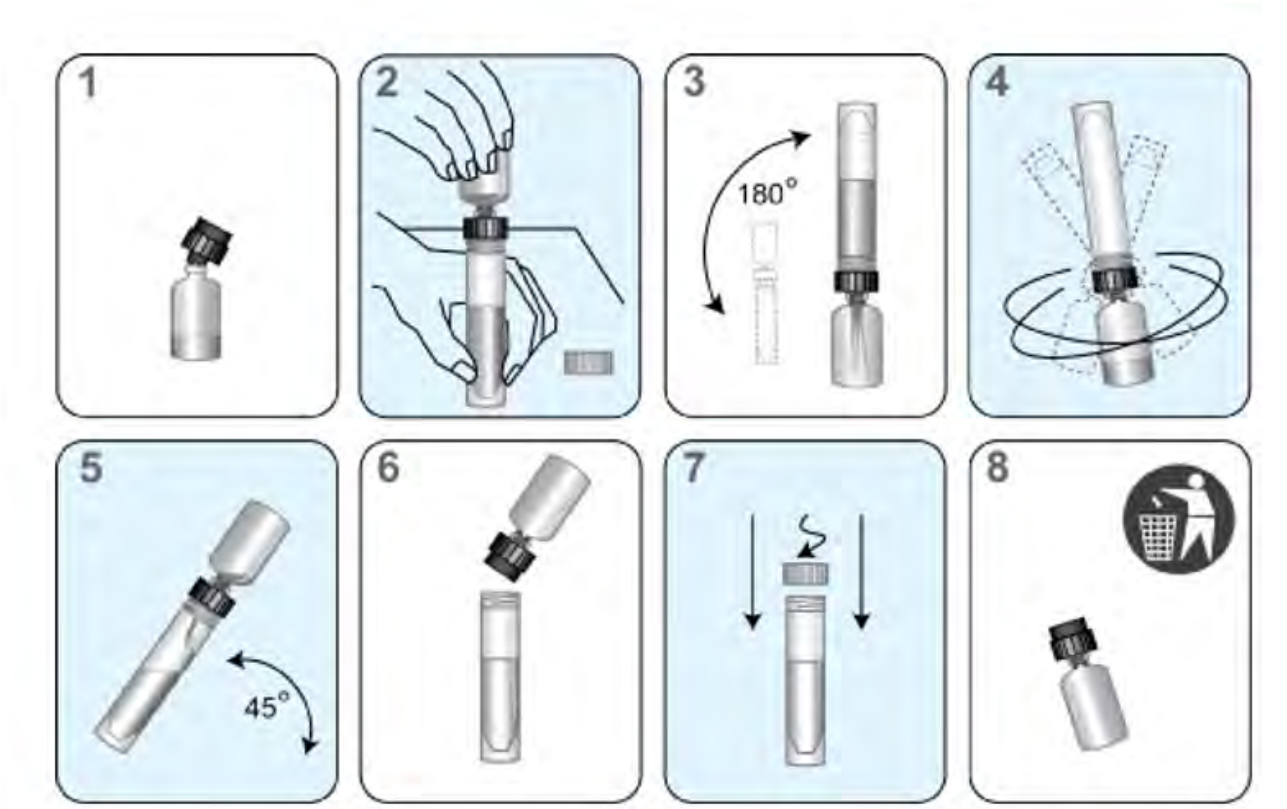


Figure 1. Panther System Reconstitution Process

2. Prepare Working Target Capture Reagent (wTCR)

a. Pair the appropriate bottles of TCR and IC.

b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents in the kit are paired.

c. Open the bottle of TCR, and set the cap on a clean, covered work surface.

d. Open the IC bottle and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.

e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.

f. Record operator initials and the current date on the label.

g. Discard the IC bottle and cap.

3. Prepare Selection Reagent

a. Check the lot number on the reagent bottle to make sure it matches the lot number on the Master Lot Barcode Sheet.

b. Record operator initials and the current date on the label. Note: Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.

C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Amplification, Enzyme, and Probe Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.

Option: The reagents may be brought to room temperature by placing the reconstituted Amplification, Enzyme, and Probe Reagents on a tube rocker set to 20 RPM (or equivalent) for a minimum of 25 minutes.

2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat the capped bottle at a temperature that does not exceed 62°C for 1 to 2 minutes. After this heat step, the Probe Reagent may be used even if residual precipitate remains. Mix Probe Reagent by inversion, being careful not to induce foam, prior to loading onto the system.

3. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents. This step is not required if reagents are loaded onto the system directly after mixing on the tube rocker.

4. Do not top off reagent bottles. The Panther System will recognize and reject bottles that have been topped off.

5. Adequate mixing of the reagents is necessary to achieve expected assay results.

D. Specimen Handling using Panther Fusion Specimen Lysis Tube or Aptima Specimen Transfer Tube.

Note: Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section before loading specimens onto the Panther system.

1. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom or use the tip of a sterile swab to eliminate the bubble.

Note: For samples transferred to the Panther Fusion Specimen Lysis Tube or the Aptima Specimen Transfer Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube.

When adequate collected specimen is added to the tube, there is sufficient volume to perform 3 nucleic acid extractions.

E. Specimen Handling using Hologic Specimen Lysis Tube or custom Specimen Lysis Tube

1. Prepare specimens per the Specimen Processing instructions in the Specimen Collection and Storage section.

Note: For samples transferred to the Hologic Specimen Lysis Tube or a custom Specimen Lysis Tube, to avoid a processing error, ensure adequate specimen volume is added to the tube. When adequate collected specimen is added to the tube, there is sufficient volume to perform 2 nucleic acid extractions

Note: When using the Aptima SARS-CoV-2 uncapped tube assay software, remove the cap from the Positive and Negative control before loading onto the Panther system.

F. System Preparation

1. Set up the system according to the instructions in the Panther System Operator's Manual and Procedural Notes. Make sure that the appropriately sized reagent racks and TCR adapters are used.

Refer to MP7.26 Panther System SARSCOV2 Assay Operator Checklist.

A. Prepare Workspace

- Clean work areas with bleach, then water, use fresh bench coat

Change Gloves

B. Prepare Reagents

- Check remaining assay reagent report -Select Reports tab, Assay Reagent Report. Select box, use keypad to enter 1 – Any kit with ≥1 test left will be displayed
- Reconstitute reagents if necessary
- Remove any current reagents and Controls from cold room.
- Inspect all reagents gently
- Allow to come to RT (~1h)
- Gently swirl to ensure homogeneity

Change Gloves

C: Complete Panther System Tasks

- Load Tips
- Load MTUs - Check:
    - All 5 tiplets are present
    - All tubes are free of debris
    - Barcodes are present, aligned on the right and undamaged

- Load Universal Fluids if required (replace complete set of A or B)
- Ensure fluid connectors are secure after replacement
- Empty Waste
    - Dispose of liquid and solid waste simultaneously
    - Decontaminate any visible liquid spills
    - Properly position solid waste cover

Change Gloves

D. Perform Maintenance

- Complete required maintenance tasks

Change Gloves

E. Prepare Samples

- Vortex checked-in specimens, aliquot 500µl of swab or aspirate specimens (or 250µL of bronchial washing or bronchoalveolar lavage specimens along with 250µL of STM) into Specimen Lysis Tube. Invert gently to mix, spin in centrifuge 1min

Change Gloves

F. Load Samples

- Verify LIS connection is enabled (two arrows on LIS icon)
- Load SARS-Cov-2 Pos and Neg controls and samples into Sample rack (controls can be with samples or on own rack), loosening lids prior to inserting into rack
- Verify barcodes are visible after removing and discarding lids
- Cover samples with Sample Retainer

G. Load Assay Reagents

Change Gloves

- Gently swirl all components ensuring homogeneity without precipitate or oiliness
- Replace into correct position of rack (match reagent to letter on rack) and correctly seated
- Remove caps, inspect reagent for bubbles or foam – pop if present
- Ensure bottles are properly seated and barcodes are visible
- Ensure Reagent Rack and TCR bottle are loaded into matching positions

Pipetting and Assay Processing

Sample tube graphic will change from:

- Green – Test Order assigned
- Yellow – Pipetting in progress
- Blue – Pipetting complete

DO NOT REMOVE A RACK UNTIL ALL SPECIMENS ON RACK HAVE BEEN PIPETTED i.e. HAVE BLUE GRAPHIC

Feed and Monitor

- Review Run Status tab for pipetting status, reagent inventory and processing times
- View Status Panel for MTU and Tip reloading requirements

Review /Manage Results

- Select and send results to LIS
- Print desired Results Reports

Unload Reagents (depending on workflow)

- Cap and appropriately store reagents – TCR MUST BE STORED AT ROOM TEMP, other reagents can all be stored at 4°C. Use fresh caps on reagents and label these with the reagent name.

2. Load samples.

Procedural Notes

A. Printing worksheet

At the start of each day, the Panther worksheet from the previous 24 hours must be printed, attached to the *MP2.140 Panther Daily Sample Check Sheet*, QC RLU values entered into Kestral, and the paperwork filed.

B. Controls

1. To work properly with the Aptima Assay software for the Panther System, one pair of controls is required. The Aptima SARS-CoV-2 positive and negative controls can be loaded in any rack position or in any Sample Bay Lane on the Panther System. Patient specimen pipetting will begin when one of the following two conditions has been met:

a. A pair of controls is currently being processed by the system.

b. Valid results for the controls are registered on the system.

2. Once the control tubes have been pipetted and are processing for a specific reagent kit, patient specimens can be run with the associated kit up to 24 hours unless:

a. Controls results are invalid.

b. The associated assay reagent kit is removed from the system.

c. The associated assay reagent kit has exceeded stability limits.

3. Each Aptima control tube can be tested once. Attempts to pipette more than once from the tube can lead to processing errors.

4. Patient specimen pipetting begins when one of the following two conditions is met:

a. Valid results for the controls are registered on the system.

b. A pair of controls is currently in process on the system.

5. All control results must be recorded on the *MP2.140 Panther Daily Sample Check Sheet* and also in Kestral.

C. Temperature Room temperature is defined as 15°C to 30°C.

D. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

E. Performing Maintenance

When performing any routine maintenance (Daily, Weekly and Monthly), the operator must log-in to their own personal account. This is to record the operator performing the maintenance. Once the maintenance is complete, log out of the personal account and log-in to the generic account.

Logging in and out can be done at any time whilst the instrument is running.

The protocol for performing maintenance is:

- Log out of the generic login [redacted]
- [redacted]
- Conduct maintenance as usual.
- Once maintenance is completed, please log out of your individual account and log in to the Generic [redacted]

39.8 Result Interpretation and Reporting

Interpretation of Results

The Panther system automatically determines the test results for samples and controls. A test result may be negative, positive, or invalid.

The possible results are reported in a valid run with result interpretations.

SARS-CoV-2 Result	IC Result	Interpretation
Neg	Valid	SARS-CoV-2 not detected.
POS	Valid	SARS-CoV-2 detected.
Invalid	Invalid	Invalid. There was an error in the generation of this result: retest sample
Neg	Invalid	Invalid. There was an error in the generation of this result: retest sample
POS	Invalid	Invalid. There was an error in the generation

of this result: retest sample

Note: Detection of internal control is not required for samples that are positive for SARS-CoV-2.

Reporting

All negative SARS-COV-2 results are reported and authorised automatically after processing is finished via result transfer to LIMS. If the interface is down or LIS connection interrupted, manual authorisation of SARS-COV-2 negative cases is performed by Laboratory Scientists.

All positive SARS-COV-2 results are to be reviewed by a Laboratory Scientist and repeated on a different assay (eg. Seegene, Cobas) to confirm the result if the RLU is <1000 or if deemed necessary (eg. If CT values are required). The Laboratory Scientist will notify hospital wards if the patient is a hospital inpatient and interim/verify for the pathologist to authorise. Routine and SOT specimens do not require notification and are interim/verified.

Mislabelled or Unlabelled Specimens

All zero-tolerance mislabelled (ID discrepancy or hospital patient with <3 identifiers) and unlabelled samples are to be tested. The CORPCR test code should be added, and the "Y" removed in the "ok to authorise" field to prevent the result from auto authorising. An "N" should be added to the SMS field. Once the result has transferred, it should be replaced with a * and the report comment replaced with a "zero tolerance" report comment (h/zerotol, see below). The RLU value should remain in the body of the report for future reference.

Report comments

SARS-COV-2 Positive

Code is corpos

SARS-COV-2 Negative

Code is corneg

39.9 Internal Quality Control

Quality Control

A run or specimen result may be invalidated by the Panther System if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new kit is loaded on the Panther system or when the current set of valid controls have expired.

The Panther system is configured to require assay controls run at an administrator-specified interval of up to 24 hours. Software on the Panther system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther system which requires a new set of assay controls be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample with the wTCR. During processing, the internal control acceptance criteria are automatically verified by the Panther system software. Detection of the internal control is not required for samples that are positive for SARS-CoV-2. The internal control must be detected in all samples that are negative for SARS-CoV-2 targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

Internal QC

An internal QC program is run at least three times a week (Tuesday, Thursday and Saturday) on an internal SARS-COV-2 specimen. "SARSCOV2 IC Panther" control specimen is stored in -80ºC freezer. Bring to room temperature then process as per swab specimen instructions above. Relative Intensity Units (RLU) are recorded in LIMS and on *MP2.140 Panther Daily Sample Check Sheet*. Significant deviations in RLU values are reported to Senior Scientists for corrective action if required.

39.10 EQAP

The assay is assessed by enrolment in the RCPA QAP program for SARS-COV-2.

39.11 Kestral Alt-9 Information

The ALT9 entry is CoronaPCR.

39.12 Records

Panther Aptima Verification Document.

39.13 Associated Documentation

MP2.140 Panther Daily Sample Check Sheet.
MP7.26 Panther System SARSCOV2 Assay Operator Checklist.
MP9.52 Aptima SARS-CoV-2 Assay

39.14 References

Aptima™ SARS-CoV-2 Assay (Panther™ System) – Instructions for Use AW-21491-001 Rev. 002

39.15 Technical Support and Troubleshooting

Hologic ANZ Panther and Fusion Instruments Technical Support Information

1. The Hologic ANZ Service Helpdesk can be contacted via one of the options below.

Phone: +61 2 9888 8000 (Australia/New Zealand)
Toll Free: 1800 264 073 (Australia), 0800 694 656 (New Zealand)
Email (non-urgent): ANZ-Support@hologic.com or pantheraufse@hologic.com

2. Guide for customers: Sending system logs before or after to logging a service-related call

Complete the following procedure for exporting system log files for Hologic ANZ Review

NOTE: Do not send logs when the system is in the Assay Processing state.
a. From the Messages screen, select Send Logs. A "Send Logs" window opens.

b. Select the start date as the day before the event
c. Select the end date as the day of the event OR as specified by Hologic Technical Support
d. Do not change the default categories selected unless specified by Hologic Technical Support.
e. Select Send to Service Support (only available to sites that have the remote connection available) – From the confirmation window, select Yes. If send to service support is not available, follow step f) instead, otherwise proceed to step i).
f. Insert a USB stick into an available USB port on the right-hand side of the instrument
g. Select the browse button at "output directory" and browse to the USB stick (typically E:)
h. Select Send to Directory The system retrieves log files, compresses the files, and exports them to Hologic Technical Support, this process can take 10-30 minutes or longer depending on the size of logs).
i. When the task is complete an operator confirmation window appears, press OK.
j. If you have used Send to Directory – You will need to press remove USB stick and remove it from the system then follow 3) Instrument EFT below.

3. Instrument EFT (enhanced file transfer) site instructions

Send electronic files (logs) to Hologic technical support for troubleshooting purposes.
a. Insert the USB stick from the Panther into a PC with internet access
b. Log onto the following site: https://eft.gen-probe.com
c. You will need the instrument serial number (left side by power cord).

[redacted]

f. File transfer:
   i. Click on the upload icon (up arrow).
   ii. Browse to the USB stick from the Panther.
   iii. Select the file ending in ".gpz"
   iv. If there is more than one file select the most recent file. Example: Panther Instrument serial number 2090001234 with logs from 1 March 2020 "PantherLogs_01234_20200301_093000.gpz"
   v. Wait until the file is showing on the main page (There is a progress bar to the left side during upload, depending on connection speed and time, it may take some time for the file to upload)
vi. Once complete logout by pressing the person icon in the top left of screen and then press logoff and notify Hologic that a log from your system has been up

46. SARS-CoV-2 Testing by cobas® 6800

46.1 Test Mnemonic

SARS-COV-2, COVID-19, CORPCR (DVIRAL)

46.2 Purpose

Intended use

cobas® SARS-CoV-2 for use on the cobas® 6800 System is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in clinician-instructed self-collected nasal swab specimens (collected on site), and clinician-collected nasal, nasopharyngeal, and oropharyngeal swab specimens from individuals who meet COVID-19 clinical and/or epidemiological criteria.

This test is also intended for the qualitative detection of nucleic acids from SARS-CoV-2 in pooled samples containing up to and including six individual samples from clinician-instructed self-collected nasal swab specimens (collected on site), or clinician-collected nasal, nasopharyngeal, and oropharyngeal swab specimens. Negative results from pooled samples should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, pooled samples should be tested individually. Specimens included in pools with a positive or presumptive positive result must be tested individually prior to reporting a result. Specimens with low SARS-CoV-2 RNA concentrations may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, recent exposures and epidemiological information. cobas® SARS-CoV-2 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and on the use of the cobas® 6800 System.

46.3 Principle

Explanation of the test

cobas® SARS-CoV-2 is a qualitative test for use on the cobas® 6800 System for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA in individual or pooled nasal, nasopharyngeal, and oropharyngeal swab samples collected in Copan Universal Transport Medium System (UTM-RT), BD™ Universal Viral Transport System (UVT), cobas® PCR Media, Edwards VTM, Copan Liquid Amies, or 0.9% physiological saline. The RNA Internal Control, used to monitor the entire sample

preparation and PCR amplification process, is introduced into each specimen during sample processing. In addition, the test utilizes external controls (low titre positive control and a negative control).

Principles of the procedure

cobas® SARS-CoV-2 is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The cobas® 6800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the cobas® 6800 software, which assigns test results for all tests. Results can be reviewed directly on the system screen and printed as a report.

Nucleic acid from patient samples and added internal control RNA (RNA IC) molecules are simultaneously extracted. Nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors, are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature. External controls (positive and negative) are processed in the same way with each cobas® SARS-CoV-2 run.

Selective amplification of target nucleic acid from the sample is achieved using target-specific forward and reverse primers for ORF1 a/b non-structural region that is unique to SARS-CoV-2. Additionally, a conserved region in the structural protein envelope E-gene were chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection sets will also detect SARS-CoV-2 virus.

Selective amplification of RNA Internal Control is achieved using non-competitive sequence specific forward and reverse primers which have no homology with the coronavirus genome. A thermostable DNA polymerase enzyme is used for amplification.

The cobas® SARS-CoV-2 master mix contains detection probes which are specific for the coronavirus type SARS-CoV-2, members of the Sarbecovirus subgenus, and the RNA Internal Control nucleic acid. The coronavirus and RNA Internal Control detection probes are each labelled with unique fluorescent dyes that act as a reporter. Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the RNA Internal Control. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

46.4 Requirements

Refer to product insert document MP9.61.

Precautions and handling requirements - Warnings and precautions

Reagent handling

- Handle all reagents, controls, and samples with good laboratory practice to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing and notify Roche.
- Roche cobas omni–Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Roche cobas® SARS-CoV-2 test kits, cobas® SARS-CoV-2 Control kit, cobas®Buffer Negative Control kit, cobas omni MGP Reagent, and cobas omni–Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow cobas omni–Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents as biohazard waste.
- If spills occur on the cobas® 6800 instrument, follow the instructions in the cobas® 6800 systems – User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Refer to product insert document MP9.61.

Transport and storage

Refer to product insert document MP9.61.

46.5 Procedure

Start system and log on

1. If the instrument is in Hibernate status, press ⏻ below the monitor.
2. Press ⏻ on the sample supply module. Once the output buffer has changed to ↓, load an empty rack tray on the output buffer to finish the initialisation.
3. Once the User Interface has loaded, choose Log on. Choose Log on and scan the datalogic quick scan onto the scan icon and log in.
4. Wait for system to enter Standby before proceeding.

Unloading amplification plates

1. On the Monitoring tab, select the instrument and then select the amplification plate drawer. Press Open drawer. Wait for the drawer to open slightly then manually open it fully.
2. Remove the amplification plates and manually close the drawer.

Emptying solid waste container

1. Select the wash/waste drawer. Press Open drawer. Wait for the drawer to open slightly then manually open it fully. Turn the solid waste container to the right side until it clicks into place.
2. Remove both solid waste bag and replace with two empty bags. Take care to ensure waste bags are flat against the sides of the container.
3. Select the button to Confirm that the waste is empty

Replacing liquid waste containers

1. With the wash/waste drawer open, pull out the liquid waste drawer.
2. Choose Unlock for the container that needs to be replaced. Wait for the click sound and wait until the status indicator next to the waste dispense cap is turned off.

3. Lift the waste dispense cap. Recap and remove the liquid waste bottle. Place empty liquid waste bottle in position, close the waste dispense cap and wait for indicator light to turn back on.

4. Push the liquid waste drawer back in.

Loading wash reagent

1. With the wash/waste drawer open, pull out the wash reagent drawer until it clicks into place.
2. Choose Unlock for the container that needs to be replaced. Wait for the click sound and status indicator light to turn off.
3. Pull out the reagent arm and turn it to the park position. Recap and remove the empty container. Load new wash reagent container. Close reagent aspiration arm and wait for indicator light to turn back on.
4. Close the wash/waste drawer.

Loading lysis reagent and specimen diluent

Note: Always bring specimen diluent and lysis reagent to room temperature before loading.

1. Select the bulk reagent drawer. Press Open drawer. Wait for the drawer to open slightly then manually open it fully.

2. Choose Unlock for the container that needs to be replaced. Wait for the click sound and wait until the status indicator next to the reagent aspiration arm is turned off.
3. Pull out the reagent arm and turn it to the park position. Recap and remove the empty container. Load new lysis reagent or specimen diluent. Close reagent aspiration arm and wait for indicator light to turn back on.
4. Close the bulk reagent drawer

Loading tip racks, reagent and control cassettes

Please note: Visually inspect reagent and control cassettes for misalignment or damage prior to loading

1. Select the reagent cassette drawer. Press Open drawer . Wait for the drawer to open slightly then manually open it fully until it clicks into place.

2. Place tip racks in magazine one at a time. Wait until barcode of each tip rack is read before loading another rack in the same magazine.
3. Place reagent or control cassette in the magazine. Wait until the cassette has been moved into the reagent storage and the system is ready to load a new cassette before proceeding.

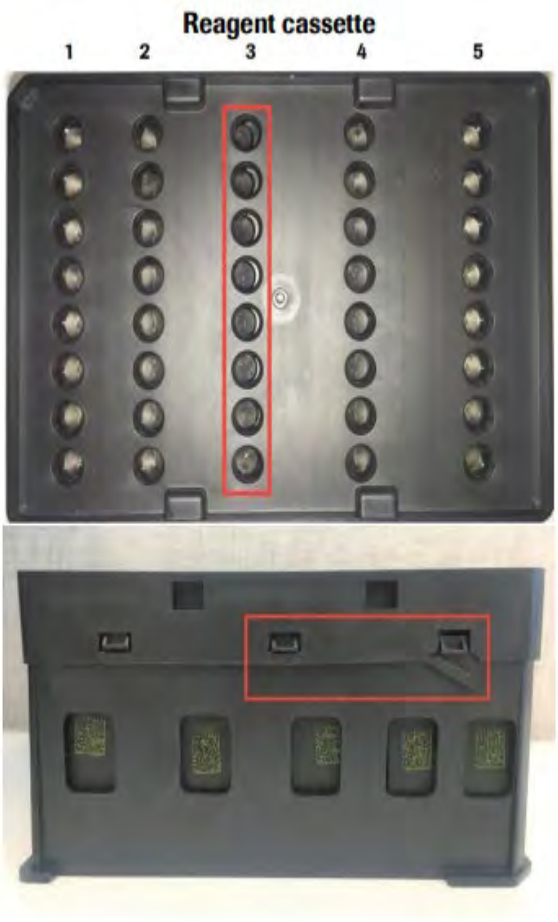
4. When all reagents and consumables are loaded, press Close drawer in the callout. Wait for the click sound, then push the reagent cassette drawer back in manually until it is fully closed.

Loading processing plates, MGP cassettes and amplification plates

Note: MGP cassette on board stability is measured from time the cassette is loaded into magazine.

1. Select the consumable drawer. Press Open drawer. Wait for the drawer to open slightly then manually open it fully until it clicks into place.
2. Load new processing plates into corresponding magazines one at a time. Wait for the barcode to be read before loading a new plate.

3. If necessary, load new MGP cassette into corresponding magazine.
4. Load new amplification plate cassettes into corresponding magazine.
5. In the callout, press Close drawer . Wait for the click sound, then push the consumable drawer back in manually until it is fully closed.



Please notify Roche if any misalignment or cracks are observed in reagent cassettes and keep reagent cassettes aside for Roche to collect. Any affected reagent cassettes will be replaced by Roche

If misalignment of control mini-racks is observed, please ensure all mini-racks are properly clipped into the control cassette prior to loading on the system.

Please note:Do not use cobas® SARS-CoV-2 reagents, cobas® SARS-CoV-2 Control Kit, cobas® Buffer Negative Control Kit or cobas omni reagents after their expiry dates.

Do not reuse consumables. They are for one-time use only.

Running cobas® SARS-CoV-2

The cobas® SARS-CoV-2 requires a minimum sample volume of 700uL of media in 13mm and ImL in the 16mm secondary tubes.

Always use caution when transferring specimens from a primary collection tube to a secondary tube.

Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.

Always use a new pipette tip for each specimen.

Ensure samples are equilibrated to room temperature prior to transfer into a cobas omni–Secondary Tube.

Sample pooling for SARS-CoV-2 testing

Pools up to 6 samples can be tested using cobas® SARS-CoV-2. Pool size is influenced by the positivity rate of SARS-CoV-2 in the testing population, and the potential risks of testing in pools.

Automated Pooling on the Hamilton STAR.

1. Follow method manual (MP4.47 Hamilton Microlab Liquid Aliquoting Robot Protocol) for detailed instructions.

Pooling from 1 to 6 samples can be done manually or automated on the Hamilton Microlab STAR instrument.

Manual Pooling.

1. Ensure each sample has sufficient volume for pooling.

2. For processing of single specimens

3. Using a calibrated filtered micropipette, carefully pipette each individual sample associated with that pool into the appropriate secondary tube to prepare the pool. Transfer 200uL of sample to Secondary Pool Tube for pool of 2-6 (i.e., for 4-Pool sample total of 800uL, 6-Pool 1200uL)

4. Ensure complete mixing after addition of all samples to the secondary tube (i.e., through vortexing/inversion). Use caution to avoid creating bubbles, foam or aerosols while mixing.

5. For manual pooling, it is recommended to visually compare the pooled sample volume in the secondary tube to a secondary tube containing the target pool volume. If the pooling tube level is less or more than the standard pool volume, then the manually prepared pool should be discarded and prepared again.

Pool result reporting and follow-up testing

Interpretation of pool results is the same as for individual results as described in the Interpretation of results section.

If the result of the pool is negative, then each constituent sample is reported as negative, and results are auto authorised.

If the result of the pool is positive or presumptive positive, then each of the constituent samples must be retested as a separate individual samples.

All positive results are sent to LIS to the covid authorisation daybook to be interim verified by scientists and authorised by pathologists using the “hot keys”.

Find the individual samples making up the positive pool for testing.

Individual test results supersede the pool result. If the result of the pool is invalid, each constituent sample should be re-tested as a separate individual sample.

Results

The cobas® 6800 Systems automatically detect the SARS-CoV-2, for each individually processed or pooled sample and control, displaying individual target results for samples as well as test validity and overall results for controls.

Negative results are reported as Not Detected and authorised by LIS.

Positive results are reported as DETECTED and crossing point values for both or either targets is transferred to LIS. A positive result is not automatically released but held and reviewed by a pathologist and authorised.

Quality control and validity of results

- One cobas® Buffer Negative Control [(-) Ctrl] and one [SARS-CoV-2 (+) C] are processed with each batch.
- A Positive SARS-COV-2 extraction QC (external QC) sample is run three times a week (Tuesday, Thursday & Saturday).
- In the cobas® 6800 software report/check for flags and their associated results to ensure the batch validity.
- All flags are described in the cobas® 6800 Systems User Guide.
- The batch is valid if no flags appear for any controls. If the batch is invalid, repeat testing of the entire batch. Validation of results is performed automatically by the cobas® 6800 software based on negative and positive control performance.

Interpretation of results - cobas® SARS-CoV-2 for System Software v1.2

cobas® SARS-CoV-2 for System Software v1.3 or higher

Display examples for cobas® SARS-CoV-2 for System Software v1.3 or higher are shown in Figure 3.

Figure 3 Example of **cobas**® SARS-CoV-2 results display for System Software v1.3 or higher

Test	Sample ID	Valid*	Flags	Sample type	Overall result*	Target 1	Target 2
SARS-CoV-2 400 µL	Swab_01	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _C1	NA	Y40T	Swab	**NA**	Invalid	Invalid
SARS-CoV-2 400 µL	Swab _B1	NA		Swab	**NA**	Negative	Positive
SARS-CoV-2 400 µL	Swab _B2	NA		Swab	**NA**	Positive	Positive
SARS-CoV-2 400 µL	Swab _D1	NA		Swab	**NA**	Negative	Negative
SARS-CoV-2 400 µL	Swab _A6	NA		Swab	**NA**	Positive	Negative
SARS-CoV-2 400 µL	Swab _E1	NA	C01H2	Swab	**NA**	Positive	Invalid
SARS-CoV-2 400 µL	Swab _A2	NA	C01H1	Swab	**NA**	Invalid	Positive
SARS-CoV-2	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
SARS-CoV-2	C161420284093009580264	Yes		SARS-CoV-2 (+) C	**Valid**	Valid	Valid

* The "Valid" and "Overall Result" columns are not applicable to sample results for the **cobas**® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns. Refer to Table 13, **cobas**® SARS-CoV-2 results interpretation, for specific instructions on test results interpretation.

Interpretation of results

The following result interpretation applies to both cobas® 6800 software version 1.2 and cobas® 6800 software version 1.3 and higher.

For a valid batch, check each individual sample for flags in the cobas® 6800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- The "Valid" and "Overall Result" columns are not applicable to sample results for the cobas® SARS-CoV-2. Values reported in these columns are not applicable and do not impact the validity of results reported within individual Target Result columns.
- Invalid results for one or more target combinations are possible and are reported out specifically for each channel.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting SARS-CoV-2 are shown below (Table 13)

Table 13 cobas® SARS-CoV-2 results interpretation

Target 1	Target 2	Interpretation
Positive	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Positive	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Detected. A positive Target 1 result and a negative Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 2, target region, or 3) other factors.
Negative	**Positive**	All Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. A negative Target 1 result and a positive Target 2 result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the Target 1 target region in the oligo binding sites, or 3) infection with some other Sarbecovirus (e.g., SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or 4) other factors. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Negative**	All Target Results were valid. Result for SARS-CoV-2 RNA is Not Detected.
Positive	**Invalid**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Detected.
Invalid	**Positive**	Not all Target Results were valid. Result for SARS-CoV-2 RNA is Presumptive Positive. For samples with a Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Negative	**Invalid**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Negative**	Not all Target Results were valid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.
Invalid	**Invalid**	All Target Results were invalid. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

46.6 Dilution Instructions

See Pooling Information in 46.8 Procedure.

46.7 Quality Control

- A Positive SARS-COV-2 extraction QC (external QC) sample is run three times a week (Tuesday, Thursday & Saturday).
- The crossing point values are recorded in LIMS and reviewed at data entry and routinely every 6 months/long term QC.

46.8 EQAP

RCPA Microbiology Molecular Coronavirus SARS-Cov-2 – 4 Surverys.

46.9 Action Results

Confirmatory testing is performed at the direction of the Microbiologist on call.

46.10 Critical Results

All positive results must be phoned through to the Microbiologist on call.

46.11 Kestral Alt-9 Information

Test Code: CORPCR (RDL: DVIRAL)

46.12 Safety Precautions

This testing platform requires the transfer of open tubes containing potentially infectious samples into the Cobas® 6800 system.

All sample transfer from primary tubes into secondary tubes should be done in a Biohazard Safety cabinet or closed enclosure.

All secondary tubes should be loaded into sample racks and rack trays and uncapped inside the cabinet.

Prior to removal from the cabinet, the staff member must be wearing full PPE and the rack tray containing uncapped specimens should be placed into a covered box.

The covered box should be carefully transferred to the bench beside the Cobas® 6800 system where the box can be uncovered, the rack tray carefully removed and loaded into the Sample Supply Module.

46.13 Records

Validation Documents Location:
G:\Pathology\Molecular\LAB\Method Validation and kit trials\Viral\2021 COBAS6800 COVID19

46.14 Associated Documentation

- MP9.61 Cobas 6800 Sars-Cov-2 Qualitative.

46.15 References

- cobas® SARS-CoV-2, Qualitative assay for use on the cobas® 6800/8800 Systems, For use under the Emergency Use Authorization (EUA) only package insert.

37. SARS-COV-2 Testing by GeneXpert.

37.1 Test Mnemonic

RDL: CORPCR

37.2 Purpose

This document describes the procedure for performing SARS-COV-2 testing on the GeneXpert instrument.

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.

Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARSCoV-2.

The Xpert Xpress SARS-CoV-2 test is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology.

The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument.

The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional.

The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability. The nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL Xpert Xpress SARS-

CoV-2 5 transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

37.3 Principle

The Xpert® SARS-COV-2 Assay, performed on the GeneXpert® Instrument Systems, is a qualitative in vitro real-time PCR test designed for the automated detection of SARS-COV-2 from swabs in Viral Transport Media (VTM).

The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform for real-time PCR detection of NG DNA. Test results are obtained in approximately 6**0 minutes**.

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/ aspirate) collected from individuals suspected of COVID-19 by their healthcare provider.

Testing of nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx.

Testing of nasopharyngeal, nasal, or mid-turbinate swab specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized to be distributed and used in patient care settings outside of the clinical laboratory environment. Results are for the detection of SARS-CoV-2 RNA.

The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx system.

37.4 Requirements, Storage and Handling

The Xpert® SARS-COV-2 Assay kit (XPRSARS-COV2-10)
The GeneXpert Instrument and Software

Store the Xpert® SARS-COV-2 Assay cartridges and reagents at 2-28 °C.

- Do not use reagents and cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the cartridge lid.
- Do not use any reagents that have become cloudy.

Proper specimen collection, storage, and transport are critical to the performance of this test.

Inadequate specimen collection, improper specimen handling and/or transport may yield a false result.

Nasopharyngeal, nasal, and midturbinate swabs and nasal wash/aspirate specimens can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2–8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems.

37.5 Procedure

37.5.1 Worklist

1. Create a worklist of the samples to test in the Laboratory Information System.
    a. Open Kestral and select **Patient Data** then **Services**.
    b. Select **Molecular Worklists**
    c. Select **Coronavirus Worksheet** and press [X].
    d. Press [P] to print out the worklist.
    e. Press [Q] to close the window and [Esc] to go back to the main menu.
    f. Print out specimen labels as required.

37.5.2 Instrument Preparation

1. Switch on the GeneXpert first followed by the computer. The reverse order is followed when shutting down the instrument.
2. Log onto the computer at the Windows Log-In Page for Cepheid Admin icon.

[redacted]

3. Click on the GeneXpert DX icon. Enter the following username and password

[redacted]

4. The GeneXpert DX System screen will appear and the computer will connect with the instrument. A self-test will be performed.

5. No further instrumentation preparation steps are required at this stage

37.5.3 Sample Preparation

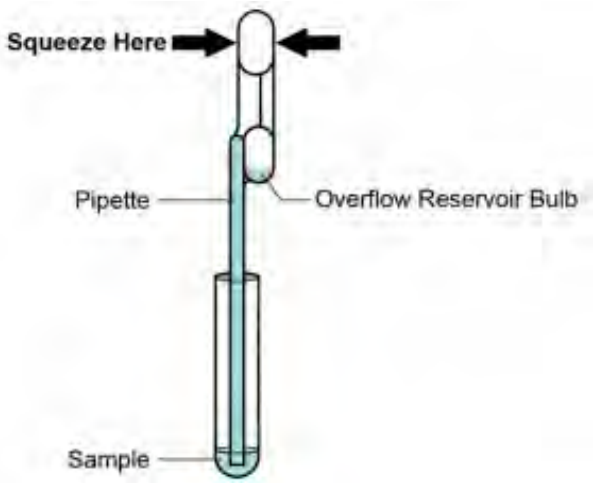
1. Swabs - Vortex swab specimen in VTM.
2. Fluids – (NPA's, ETT's, bronchial washings and lavages). Add 600ul of specimen to 3mLs of sterile saline in a 5ml screw top tube. Vortex.

37.5.4 Preparing the Cartridge

Important: Start the test within 30 minutes of adding the sample to the cartridge.

1. Collect a single Cepheid Xpert® **SARS-COV-2** Assay cartridge and Transfer Pipette from the kit located in the Molecular Pathology Section in Clean Cold Room 10.4.57.

2. Place the cartridge, pipette and samples to be tested in a clean biohazard cabinet or workbench. It is not necessary to bring the cartridge to room temperature before use.



3. In the cabinet or workbench, open the cartridge outer packaging and place the cartridge on the deck. Place a specimen sticker on the side of the cartridge. Check for a small node of plastic left after manufacture on the bottom of the cartridge. Remove the excess plastic node as required.

4. Open the cartridge lid.

5. In the cabinet or workbench, fill the transfer pipette to the *MARK* on the pipette shaft (Figure 1) with the specimen.

6. Using the filled pipette, transfer the entire sample contents to the "S" opening of the Xpert® SARS-COV-2 Assay cartridge (Figure 2). Discard pipette.

7. Close the cartridge lid. Take the cartridge to the GeneXpert instrument. Do not shake or invert the cartridge during transit and place the cartridge on the bench in an upright position.

   The test is invalidated if the cartridge is dropped or shaken prior to testing.

37.5.5 Starting the Test

Important: Before starting the test, ensure the Xpert® SARS-COV-2 assay definition files are imported into the software. Assay definition files are on the supplied CD.

1. At the GeneXpert DX System computer screen, select Create Test. Scan barcode on the cartridge.

2. In the Sample ID box, enter the patient ID details manually or scan the specimen label. The sample ID is associated with the test results and is shown in the View Results window and all the reports.

3. From the Select Assay drop-down menu, select the **Xpert SARS-COV-2** assay to be run. Select the assigned module to be used. Add further information to Notes as needed. Click Start Test.

4. Open the instrument module door with the blinking green light and load the cartridge. HOLD the door CLOSED until the door is held closed by the instrument. The run will start and run for 60 minutes.

5. The test starts and the green light stops blinking. When the test is finished, the light turns off. When the system releases the door lock, open the module door and remove the cartridge. Dispose of the used cartridges in the hazardous waste bin.

37.5.6 Quality Control

Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC) and a Probe Check Control (PCC).

An external Quality Control (QC) sample is tested every month and for every new kit batch, an external QC sample must be tested and the results valid before the new batch can be used. SARS-COV-2 results are entered in Kestral Utilities section (refer to document MP1.08). Report results are kept in the GeneXpert QC folder.

Sample Processing Control (SPC)—Ensures the sample was correctly processed. The SPC verifies that binding and elution of target NA have occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay. The SPC should be positive in an analyte negative sample and can be negative or positive in an analyte positive sample. The SPC passes if it meets the validated acceptance criteria.

Sample Adequacy Control (SAC)—Ensures that the sample contains human cells or human DNA. This multiplex assay includes primers and probes for the detection of a single copy human gene. The SAC signal is only to be considered in an analyte negative sample. A negative SAC indicates

that no human cells are present in the sample due to insufficient mixing of the sample or because of an inadequately taken sample.

Probe Check Control (PCC)—Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.

Interpretation of Results

The results are automatically interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.

The Xpert SARS-COV-2 Assay provides test results according to algorithms shown below. The final result is shown in the Test Result window. Screenshot examples of the Test result windows for various result scenarios are shown in Figure 4 – 8. Further details on result interpretations are given in Table 2.

Xpert Xpress SARS-CoV-2

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass

Xpert Xpress SARS-CoV-2

Result	Interpretation
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check – PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

37.5.7 Failed Results – Error Messages

In all cases of failed results, repeat the assay according to the procedure described above.

37.5.8 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to instructions in the following section titled “Retest Procedure”.

- An INVALID result indicates that the controls SPC failed. The sample was not properly processed or PCR is inhibited.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.

- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

37.5.9 Retest Procedure

If the test fails again, the test is invalid and an INDETERMINATE result is reported. See procedure MP5.02 Section 2.7 for reporting INDETERMINATE cases.

37.6 Reporting Results

37.6.1 Laboratory Information System

All results need to recorded in the Laboratory Information System (LIS).

1. Log in to the Kestral LIS using your designated username and password.
2. At the main menu, select Patient Management and Services.
3. Select Molecular Worklist and then Viral and Bacterial Daybook (Select X)
4. Sort the worklist by Request Order (Use arrows and the Space Bar to select then press Enter)
5. Select the appropriate sample using the arrow press Enter to open the record. Select E to edit the record and enter the data.
6. Type in the site information noted on the worksheet. Press the enter key to move to the result field. Type in ND (not detected) or D (detected) then press the F2 key to expand the result.
7. Press F10 to open the Silent comment field. Enter the date, assay, the number of replicates tested and the result with crossing points if positive. If any replicates are negative, enter these too. Enter your initials.
8. Press F10 to exit the Silent comment field. This result/comment must also be entered into the Silent comment field.
9. Press F9 to exit the result data entry and H (HOLD) to save the data and close the episode.
10. Open the Coronavirus Sars-CoV-2 PCR test result episode and press E to edit. Use the Enter key to move down to the Silent comment field and enter the exact result into the silent field

37.6.2 Authorising Results

The results and data entry must be checked by a senior scientist before release.

The following needs to be checked by the authorising officer.

1. Assay results are deemed acceptable and the data transcription to the worksheet is accurate.

2. The data entry of the result and patient details from the referral are correct.

37.7 Method Limitations

• Performance of the Xpert Xpress SARS-CoV-2 test has only been established in nasopharyngeal swab and nasal wash/aspirate specimens. Use of the Xpert Xpress SARS-CoV-2 test with other specimen types has not been assessed and performance characteristics are unknown.

• Oropharyngeal, nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic testing for SARS-CoV-2 for additional information.

• A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.

• As with any molecular test, mutations within the target regions of Xpert Xpress SARSCoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.

• This test cannot rule out diseases caused by other bacterial or viral pathogens.

The Xpert SARS-COV-2 Assay provides qualitative results only.

37.8 Associated Documentation

MP1.08 – QC and QA Specimens and Procedures

38. SeeGene Allplex SARS-COV-2 Assay

38.1 Test Mnemonic

SeeGene Allplex SARS-COV-2 Assay.

38.2 Purpose

The SeeGene AllplexTM SARS-CoV-2 Assay is in vitro diagnostic medical device designed for qualitative detection of SARS-CoV-2 with real-time reverse transcription PCR from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known by the provisional name 2019 novel coronavirus (2019-nCoV), is the cause of the respiratory coronavirus disease 2019 (COVID-19).

Taxonomically, it is a strain of the severe acute respiratory syndrome-related coronavirus (SARSr-CoV), a positive-sense single-stranded RNA virus. It is contagious in humans, and the World Health Organization (WHO) has designated the ongoing pandemic of COVID-19 a Public Health Emergency of International Concern.

SARS-CoV-2 is believed to have zoonotic origins. It has close genetic similarity to bat coronaviruses, suggesting it emerged from a bat-borne virus. An intermediate animal reservoir such as a pangolin is also thought to be involved in its introduction to humans. Chinese scientists first isolated SARS-CoV-2 from patients in Wuhan, China who had had pneumonia of unknown cause in December 2019.

38.3 Principle

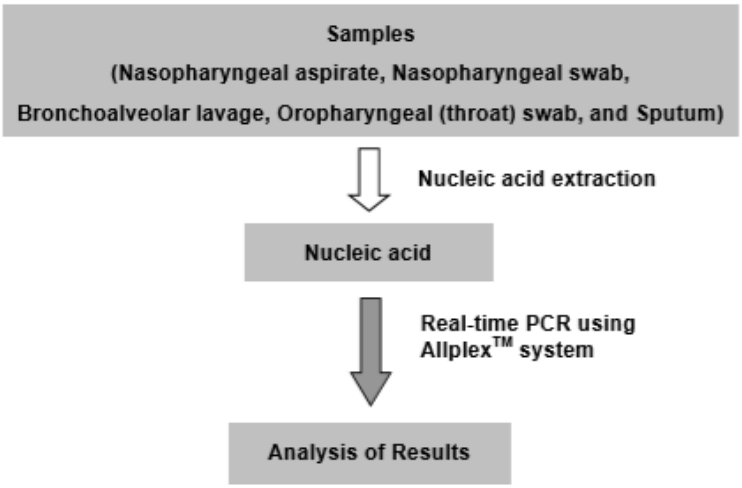
The SeeGene Allplex™ SARS-CoV-2 Assay is a multiplex real-time RT-PCR assay that enables simultaneous amplification and detection of target nucleic acids of E gene, RdRP gene, S gene, and N gene with Internal Control (IC).

The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

An exogenous gene is used as Internal Control (IC) to monitor the whole process of sample collection, nucleic acid extraction and to check for any possible PCR inhibition.

To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ SARS-CoV-2 Assay. The UDG-dUTP system is

commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.



38.4 Patient Preparation

Not applicable.

38.5 Requirements

The reagents in one kit contains 100 tests.
Order information: Cat. No. RV10248X
All components of Allplex™ SARS-CoV-2 Assay should be stored at ≤ -20°C.

Allplex™ SARS-CoV-2 Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	SARS2 MOM	500 µL	Oligo Mix : - Amplification and detection reagent
ENZYME	EM8	500 µL	- RTase - DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	SARS2 PC	50 µL	Positive Control (PC): - Mixture of pathogen and IC clones
CONTROL IC	RP-V IC 2	1,000 µL	Exogenous Internal Control (IC)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

All components are stable under recommended storage conditions until the expiry date stated on the label.

The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

Materials required but not provided

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and Sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Low-Profile 0.2 mL 8-Tube Strips without Caps (white color, Cat. No. TLS0851, Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Hard-Shell® PCR plates 96-well WHT/WHT (Cat. No. HSP-9655, Bio-Rad)
- Hard-Shell® PCR plates 96-well WHT/WHT, Barcorded (Cat. No. HSP-9955, Bio-Rad)
- Nucleic Acid Extraction System (see Nucleic Acid Extraction)
- Ice Maker
- Desktop centrifuge
- Vortex mixer
- CFX96™ Real-time PCR Detection system (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)

Information

- For in vitro diagnostic use only.
- Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.
- This product is only for use with Microlab NIMBUS IVD, Microlab STARlet IVD, Seegene NIMBUS and Seegene STARlet in maximum 5 separate runs.
- This test has been validated for the following specimen types: nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum. This test has not been validated for any other types of specimens.
- Store RNA samples at ≤ -20°C until use and keep on ice during use.
- Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer time.
- Workflow in a laboratory should proceed in a unidirectional manner.
- Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.
- Supplies and equipment must be dedicated to working areas and should not be moved from one area to another.
- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
- Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.
- Do not pool reagents from different lots or from different tubes of the same lot.

- Do not use the product after its expiry date.
- Do not reuse all disposable items.
- Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.
- Be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive controls. To prevent contamination of reagents, the use of filter-tips is recommended.
- Use separated and segregated working areas for each experiment.
- To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.
- Store positive materials separately from the kit's reagents.
- Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components including product residuals and packaging can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.
- Expiry date is 8 months from the date of manufacture at ≤ -20°C. Please refer to label for the expiry date.
- Seegene NIMBUS and Seegene STARlet are the same equipment as the Microlab NIMBUS IVD and Microlab STARlet IVD, respectively although the manufacturers are different from each other. Since there are no hardware changes on the devices, the test results are the same for those.
- The brand name of "CFX96™ Real-time PCR Detection System-IVD" has been changed to "CFX96™ Dx system". Since there are no hardware changes on the systems, it will be expected to obtain the same results from both systems.
- "CFX Manager™ Dx Software v3.1" is an upgrade version of "CFX Manager™ Software-IVD v1.6". The upgraded software includes enhancements to the "Run" menu. These enhancements do not impact the results of data analysis; therefore, the results will be the same.
- This kit is a qualitative in vitro test for the single or multiple detection of 4 types of gene (E gene, RdRP gene, S gene, and N gene).

Specimen Collection, Storage, and Transport

Note: All samples should be treated as potentially infectious materials.

Only permitted are those sample materials, which are collected, transported, and stored by attending strictly to the following rules and instructions.

Note: To ensure high quality of samples, samples should be transported as fast as possible at indicated temperature.

Specimen Collection

Nasopharyngeal aspirate, Nasopharyngeal swab, Bronchoalveolar lavage, Oropharyngeal (throat) swab specimens are routinely examined for common respiratory pathogens.

Obtaining respiratory specimens may be difficult in some patients. In such cases, nasopharyngeal swabs may be collected.

Sputum samples may also be processed by this test.

Specimen Storage & Transport

For Nasopharyngeal aspirate, Nasopharyngeal swab, Bronchoalveolar lavage, Oropharyngeal (throat) swab and Sputum – Samples are stable at 2~8°C for 3 days*.

Note: Performance may be affected by prolonged storage of specimens. - Specimens should also adhere to local and national instructions for transport of pathogenic materials.

* Duration: The time period from the specimen collection to the final test (includes transport and storage of specimens prior to tests).

Nucleic Acid Extraction

Follow procedure MP5.04. Add internal control to the specimen as detailed below.

Internal Control

The internal control included in the kit allows the user not only to verify the nucleic acid extraction procedure but also to identify any PCR inhibition.

Aliquot 10 µL of RP-V IC 2 tube manually into samples or via Hamilton robot into the extraction cartridge prior to extraction on the MP96, MT-PREP or other extraction system.

38.6 Calibration

A **Positive Control QC** sample is supplied by the kit and must be included in every run.
A **PCR negative water QC** sample from the kit is included in every run.
An **extraction negative QC** sample from the extraction is included in every run.
A **Positive SARS-COV-2 extraction QC** sample is included in every extraction and test run.

38.7 Calibrator Traceability

Crossing point values for positive QC samples are recorded in LIMS and reviewed every six months.

38.8 Procedure

A: Set up of Hamilton Star Robot for pooling

See **MP4.47 Automated Liquid Handling: MICROLAB STAR Robot** for instructions on how to prepare a Seegene cartridge for extraction on the MP96 and Chemagic platforms. Currently, only

singles or pools of two are validated for aliquoting on the Hamilton robot for the Seegene and Chemagic assays.

On completion of the Hamilton run, remove the plate, and manually assess liquid levels in the sample and control wells. Fill in a cover sheet and include a copy of the first pool sticker for the run. Locate the LIMS file for the run on the desktop and open to format:

1. Select all samples and two extra columns to the right. Widen the two columns slightly to accommodate handwritten notes when printed. Name one column "**Verifed**" and the other "**Comments**".

2. Select the Borders icon and select "**All Borders**".

3. Select the "**Print Area**" tab at the top and select "**Set Print Area**".

4. Select the "**Print Tiles**" icon, then "**Header/Footer**" → "**Custom Header**"→Select the green icon "**Insert File Name**". Then select ok twice to exit the window.

5. Print this document and attach to the cover sheet.

B: Extraction on the Roche MP96 Extraction Machine

- **Computer Setup:**
    - Open the MP96 software on the desktop. Log on [redacted]

    - Select the **Overview** tab, then the **Enter Order** icon. On the left side, click the dropdown menu under **MP96 Kit Name.** Select the "**DNA/Viral NA SV 2.0**" kit. click the dropdown menu under **Protocol**, select the "**Viral NA Universe SV 4.0**" Kit. Select the sample volume as **200uL** and the elution volume as **50uL**.

    - Now enter the sample names on the sheet: For a cartridge prepare on the Hamilton robot, a template can be used (such as "blank" for all fields). Change sample number 1 to the first pool number (the one stuck on the MP96 cartridge). Enter "Cor Ext" on well 93 and "Neg Ext" on well 94. Wells 95 and 96 stay blank.

    - Now save the run (top right save icon) with a "***PLXxxxx yyyymmdd hhmm***" format. The machine can now be set up for the run saved. Select the arrow on the bottom right after saving to proceed to the layout of the deck.

- **Roche MP96 Setup** (*A full run will need a Roche kit with at least 96 tests left*):
    - Place an empty cartridge in the last slot of the second and third drawer.
    - Place the MP96 kit cassettes (2) in one of these drawers, the second drawer will just have the empty cartridge.
    - Place the magnetic beads in the first, narrower, drawer in the first two slots.
    - Ensure there are enough tips in the fourth drawer along for a full run – at least two full tip racks of 96 tips.
    - Place an MP96 output plate in the fifth drawer, at the front.
    - Ensure the sixth drawer is free of used tips – empty tip racks should replace the full used racks (plenty of them in the cupboard next to the machine).
    - Close the machine so it can initialise before adding the cartridge with samples in it from the Hamilton.
    - Carefully place the prepared cartridge in the last slot of the fifth drawer. **Please ensure correct orientation** (ie: 1A is top left).
    - Close the drawer and the MP96 load flap. Allow machine to initialise.
    - On the computer software, the layout of the deck can be assessed as the machine initialises. The fields should turn green as they are initialised.
    - When the machine has finished initialising it will loudly "click" and the "**start run**" icon on the top right of the computer screen will become green. Press this button to start the run. The progress screen will show how far along the run is as it progresses.
    - One run takes approximately 60 minutes.
    - When the run has completed, the results of the run can be checked as indicated on the screen "Check Last Results". The whole plate should be green.
        - If error F1 – run PCR and carefully check results. Repeat extraction if IC insufficient or controls fail.
        - If Low Vol error, run specimen(s) on PCR and check results carefully. Repeat if IC not satisfactory.

C: Prepare the real-time PCR Mastermix

Note: The BioRad CFX-96 plates and caps must be used. Aerosol resistant filter tips and tight gloves must be used when preparing One-step RTPCR reactions. Use extreme care to prevent cross-contamination. Completely thaw all reagents before use. Keep cool once thawed. Briefly centrifuge reagent tubes to collect residual drops inside of the cap.

5 µL SARS2 MOM
5 µL EM8
5 µL RNase-free Water

15 µL Total volume of Mastermix

Note: Calculate the total amount of each reagent needed based on the number of reactions including samples and controls. One full run of 96 samples will require 540uL of each of the three reagents

- Remove the Seegene kit out of freezer when the MP96 run is 50% progressed and leave in the clean room cabinet to thaw. Bring the IC (orange lid) and Kit Positive (green lid) out into the lab to thaw. Remove the sticker and place on cover sheet.

- Prepare the Seegene Mastermix in the clean room in an amber tube using 540uL each of the RNAse Free Water, MOM and EM8. Vortex.

- Using the electronic pipette, pipette 15uL of mastermix into each well of a blank BioRad CFX-96 plate as required using the “15uL MULTI” program. The “15uL MULTI” program pipettes can dispense 16 wells worth of master mix. Discard the pipette after each use. The plate must be placed on an ice block for this process

- Once the plate has been pipetted, place a tissue over the plate. Take the plate, ice block and remaining mastermix (in the amber tube) out into the DNA lab PCR setup corner.

- Using the multi-channel pipette and yellow small pipette tips, press once to Home the pipette, then select the program “**CORSEEGENE**”, which pipettes 5uL in 8 channels.

- **Prepare Row 12 first: Pipette 5uL of RNAse Free Water into well 95, and 5uL of KIT QC (green tube) into well 96 using the single 5uL pipette**. Then pipette the SIX remaining samples using the multi-channel pipette. Once this is done, place a cap over that row and change gloves before pipetting the rest of the plate.

- Pipette each of the remaining 11 rows from the MP96 output plate into the Seegene plate. **Be careful that sample is pipetted from the Seegene plate into the 96 plate only and ensure the correct rows are being pipetted.** Place the plastic caps on the rows once sample has been added.

- Briefly centrifuge the prepared plate before proceeding immediately to the thermocycler to set up the PCR reaction.

D: Thermocycler Run Setup and CFX/LIMS data files

- Switch ON the CFX96 Thermocyclers and Computer.

- Press the Hatch button on one CFX96 Thermocycler to open the Hatch. Wait for the Hatch to open completely and load the PCR Plate into the thermal plate of the Thermocycler. Ensure correct orientation.

- Press the Return Hatch button to close the hatch.

- Open the **SARS-COV-2 + MENG-B Protocol** located on the desktop. Press ok on the protocol layout page, this may need to be dragged to the left to reveal the "ok" button.

- Select the "Start Run" tab on the top left of the window.

- Select which thermocycler is being used (Atom or Jerry) on the software and press "**Start Run**". Save the file: Desktop → Experiments →Year/Month/Day folder. Note the name of the run on the coversheet paperwork.

- Select the Real Time Status tab, then the **View/Edit Plate** option. Then select **Tools → Spreadsheet View.** Click the number column twice to orientate the plate correctly. Import the correct CFX file for the run from the Hamilton P drive.

- On this window, scroll to the bottom of the page and name the last two blank wells: NEG PCR in well 12G and KIT QC in well 12H, then press "**Exit Spreadsheet View**".

- Set the positive and negative controls by selecting the appropriate well and designating the controls from the first dropdown menu to the right and then press ok:

    - CorExt = Positive Control
    - Neg PCR = Negative Control
    - Kit QC = Positive Control

(Note that the Neg Ext is run as a normal sample, it is not a negative pcr control).

- Allow the run to finish: 1 hour and 43 minutes.

E: Reviewing and Releasing Results

- Once the run has completed, the "Data Analysis" window will be visible with the raw run data (*.pcrd file).
- Select "Tools" and then "SeeGene Export".
- At the "Browse for Folder" window, the new export file must be saved in the corresponding "Day Directory" at **C:\Desktop\Experiments\YYYY SARSCOV2\YYYYMM xxx\"DD".** Exporting creates two new folders called QuantStep4 and QuantStep5. These are required for analysis by the Viewer software. Export all runs of the day to the same "Day Directory". Do not export directly to the QuantStep4 or QuantStep5 directories.
- Close the "Data Analysis" window and BIORAD CFX Manager software if appropriate.
- Select "SAVE" to save changes to the (*.pcrd) file to the corresponding "Day Directory".
- Close the CFX manager software if not being used.
- Open the "Seegene Viewer for Real Time Instruments V3" (Red Icon) located on the desktop.
- In the Viewer, go to "FILE" then "OPEN" and select the appropriate run (from **C:\Desktop\Experiments\YYYY SARSCOV2\YYYYMM xxx\DD**[Run File.xlsx].
- Run data is shown in the window. Check POS and NEG QC samples are valid. To check validity, the PCR run PC (Positive Control) and NC (Negative Control) are valid when the following criteria are met:
    - When the criteria aren't met, the run is considered invalid. Invalid results are not interpreted or reported and the assay is repeated.

Positivity is determined according to the table below.

Analytes	C_t value	Result
Targets	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)
IC	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)

- Highlight any positive pools on the run worksheet after determining the validity of run and proceed with entering results in LIMS by interface (HL7) or manual data entry. Note any low positives or positives with less than three targets detected in the comments section of the paperwork.

- To upload results to LIMS, select all samples check boxes and select FILE, HL7 and check that MLLP and All Samples is ON and press SEND. A message will appear with a "No Response" message which can be ignored and OK selected.

- To carry out conditional formatting and print a copy of the results, select "File" then "Export", and save on a USB stick. Remove USB and take to a computer with printing capability. Open the file in Excel:
    - Delete the [sample], [patient] and [comment] columns.
    - Highlight the relevant cells for printing
    - Select "Page Layout" → Print Area → Set Print Area
    - Select "Print Tiles" → Header/Footer → Custom Header → Insert File Name (icon with green box)

Control	Seegene Viewer Result							
	FAM (C_t)		Cal Red 610 (C_t)		Quasar670 (C_t)		HEX (C_t)	Auto Interpretation
	E gene		RdRP/S gene		N gene		IC	
SARS2 Positive Control	≤ 40		≤ 40		≤ 40		≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

- On the same window, select "Page" → Fit to 1 page wide and [blank] tall
- Format borders to add borders to cells → "All Borders"
- Conditional Formatting:

- Select all cells and conditionally format (Highlight Cell Rules → Text that contains [+] and repeat with [sars].

▪ Print Paperwork and staple to coversheet and run sheet.

Result Interpretation

Results are interpreted by the software according to the following table.

Target Result			IC Result*	Auto-interpretation	Description
E gene	RdRP/ S gene	N gene			
+	+	+	+/-	SARS-CoV-2 Detected	All Target Results were valid. Result for SARS-CoV-2 RNA is detected.
+	-	+	+/-		- All Target Results were valid. Result for SARS-CoV-2 RNA is detected. - Negative target results are suggestive of 1) A sample at concentrations near or below the limit of detection of the test, 2) A mutation in the corresponding target region, 3) Other factors.
-	+	+	+/-		
+	+	-	+/-		
-	+	-	+/-		
-	-	+	+/-		
+	-	-	+/-	Presumptive positive	- All Target Results were valid. Result for Sarbecovirus RNA is detected. Result for SARS-CoV RNA is Presumptive Positive. - Negative target results are suggestive of 1) A sample at concentrations near or below the limit of detection of the test, 2) A mutation in the corresponding target region, 3) Other factors. - Repeat test with more nucleic acid (up to 10ul) - For samples with the same result on the repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2

					and other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
-	-	-	+	**Negative**	All target results were valid. Result for SARS-CoV-2 RNA is not detected.
-	-	-	-	**Invalid****	- Results suggest inadequate specimen collection or processes (*e.g.,* no exogenous IC added) or the presence of PCR inhibitors. - Repeat the test from the nucleic acid extraction using another aliquot of the original specimen. - If the same result is shown in the diluted nucleic acid, please collect samples again.

* High level of target nucleic acids may cause interference in Internal Control detection and readout. Invalid IC signal does not indicate that the positive results for targets are invalid.

** See TROUBLESHOOTINGS section for the detailed instruction.

Reporting

All negative SARS-COV-2 results are reported and authorised automatically after result transfer to LIMS. If necessary, manual authorisation of SARS-COV-2 negative cases is performed by Laboratory Scientists.

All positive SARS-COV-2 results are to be placed into interim/verify by the laboratory scientist for the Microbiologist to review, authorise, and notify. Any low positive results (<3 targets detected) must be repeated on at least one other assay. These are to remain in verify only until all results have been returned. Positive results from hospital inpatients must be reported to the relevant ward by the Laboratory Scientist and the result placed in interim/verify.

Mislabelled or Unlabelled Specimens

All zero-tolerance mislabelled (ID discrepancy or hospital patient with <3 identifiers) and unlabelled samples are to be tested. The CORPCR test code should be added, and the “Y” removed in the “ok to authorise” field to prevent the result from auto authorising. An “N” should be added to the SMS field. Once the result has transferred, it should be replaced with a * and the report

comment replaced with a “zero tolerance” report comment (h/zerotol, see below). The ct values should remain in the body of the report for future reference.

Report comments

SARS-COV-2 Positive

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, has been DETECTED.

Please refer to Public Health and Infection Prevention and Control advice regarding patient management and management of contacts.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

SARS-COV-2 Indeterminate

An INDETERMINATE result was obtained.

This may be due to low numbers of the novel coronavirus in the specimen or non-specific amplification of related viruses.

Interpretation of the results should be based on epidemiological risk and clinical features. Consideration should be made to repeat specimen collection. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

SARS-COV-2 Low Positive Specimen

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was DETECTED at very low levels.

This may represent very early infection or viral RNA shedding from a past infection. Please correlate with history and ONLY submit a repeat swab for PCR if necessary.

SARS-COV-2 Negative

Coronavirus (SARS-CoV-2), the causative agent of COVID-19 was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimen was taken early in the illness, then repeat specimen collection and testing may be indicated. Lower respiratory tract specimens appear more sensitive than upper respiratory tract specimens in hospitalised patients with evidence of pneumonia.

If the patient is in self-isolation or quarantine for a defined period of time due to overseas travel, local travel to hot spots or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt the person from completing the required period of isolation or quarantine. For further details please refer to the local Public Health Department guidelines (covid.act.gov.au).

For further advice on regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not yet been assessed in this laboratory by NATA according to NPAAC guidelines.

SARS-COV-2 Test Rejected

This specimen has not been tested for coronavirus (COVID-19) since there was insufficient information on the test request form to ascertain whether this patient met the suspect case definition at the time of specimen collection. The suspect case definition, as outlined by the ACT Chief Health Officer, is available at:
https://www.health.act.gov.au/health-professionals/chief-health-officer-alerts

This specimen has been stored. If there are important reasons for testing to be performed on this sample or if there is additional clinical information, please contact the Clinical Microbiologist via Canberra Hospital switchboard on 5124 0000 to discuss testing.

SARS-COV-2 Technical Issue

NOTE: Due to various technical issues that have arisen in the testing of this specimen, no valid PCR result can be reported.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

SARS-COV-2 Unlabelled Specimen

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected in this specimen however the specimen was received completely unlabelled therefore the laboratory cannot verify that it belongs to the person on the request form.

ACT Pathology can therefore take no responsibility for patient identification of this report. A clinical judgement will be needed by the referring doctor as to whether or not to act on this Pathology result.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens. If this patient is in self-isolation or quarantine for a defined period of time due to travel overseas, travel to a declared COVID hotspot or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department. For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

SARS-COV-2 Zero Tolerance Mislabelled/Unlabelled Specimens

Tests requested have stars and no results because the specimen or request form received for this request had a misidentification incident associated with it regarding the patient's unique identifiers.

In compliance with the Canberra Health Services Zero Tolerance policy, this request has been rejected and the specimen will need to be recollected with a new request form.

National standards require all Pathology specimens and their request forms to be correctly and accurately labelled with a minimum of three unique patient identifiers.

38.9 Dilution/Concentrating Instructions

Not Applicable

38.10 Calculations

Not Applicable

38.11 Units

Not Applicable

38.12 Internal Quality Control

Manufacturer QC and in-house QC are included in each run and crossing points are recorded in LIMS. Outliers are reviewed at the time and all data are reviewed half-yearly.

38.13 EQAP

The assay is controlled by participation in the RCPAQAP PHLN: SARS-CoV-2 Quality Assurance Program.

38.14 Method Limitations

Specificity

The high specificity of Allplex™ SARS-CoV-2 Assay is ensured by the oligos designed specifically for the targets of interest. Allplex™ SARS-CoV-2 Assay was tested by the manufacturer for cross reactivity to 48 different pathogens, and PCR amplification and detection were only identified for the specified targets.

Sensitivity

The sensitivity of Allplex™ SARS-CoV-2 Assay was determined by the manufacturer using target plasmid DNAs spiked into samples (from 103 to 100 copies/reaction). Detection limit for Allplex™ SARS-CoV2 Assay was 50 copies/reaction.

Reproducibility

The reproducibility of Allplex™ SARS-CoV-2 Assay was evaluated between sites, product lots, experimenters, and time points using 3 concentration samples of each analyte by the manufacturer. CV values met criteria of less than 10%. The results were satisfied with the criteria set above, thus confirming the reproducible performances of Allplex™ SARS-CoV-2 Assay.

Interfering substances

There was no effect on the result by adding human genomic DNA in terms of non-specific detection or inhibition on target amplification. Based on the results, human genomic DNA had no effect on Allplex™ SARS-CoV-2 Assay results.

38.15 Safety Precautions

Good laboratory practice and standard hazard prevention are necessary for this procedure. The specimen and pathogen must be treated as biological hazards and appropriate PPE must be worn. The procedure can only be performed by competent personnel.

38.16 References

Instructions for use: Seegene Allplex™ SARS-CoV-2 Assay (Cat.No. RV10248X) version 1.0.

37. SARS-CoV-2, Flu and RSV Testing by GeneXpert

37.1 Test Mnemonic

RDL: CORPCR

37.2 Purpose

This document describes the procedure for performing **SARS-COV-2, Flu (Flu A1, A2 and B) and RSV** testing on the GeneXpert instrument utilising the Cepheid 'Quad Test' **SARS-CoV-2/Flu/RSV** GX cartridge, Single **SARS-COV-2** GX cartridge and the **Flu/RSV** GX cartridge.

The Xpert Xpress 'Quad' **SARS-CoV-2/Flu/RSV** test is a multiplexed real-time RT-PCR test intended for the simultaneous, qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA in either nasopharyngeal swab, nasal swab or nasal wash/aspirate specimens collected from individuals suspected of respiratory
viral infection.

The Xpert Xpress '**Single' SARS-CoV-2** test is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

The Xpert Xpress **Flu/RSV** test is a molecular in vitro diagnostic test that aids in the detection and diagnosis of Influenza/RSV and is based on widely used nucleic acid amplification technology. The test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of Influenza/RSV RNA in upper respiratory specimens. The Xpert Xpress Flu/RSV Assay detects Influenza A and Influenza B and Respiratory Syncytial Virus. There are 2 targets for Influenza A (Flu A1 and Flu A2) and single targets for Influenza B and Respiratory Syncytial Virus.

37.3 Principle

The Cepheid Xpert Xpress Assays are performed on the GeneXpert® Instrument Systems, are a qualitative in vitro real-time PCR test designed for the automated detection of SARS-COV-2, Flu (Flu A1,A2 and B) and RSV, from swabs in Viral Transport Media (VTM).

The nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL of VTM transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

Test results are obtained in approximately:

- 30 minutes for Flu/RSV
- 50 minutes for SARS-CoV-2/Flu/RSV
- 60 minutes for SARS-CoV-2

The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert Xpress cartridges includes reagents for the detection of RNA from SARS-CoV-2 and Influenza/RSV RNA in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens. The RNA is generally detectable in upper respiratory specimens during the acute phase of infection.

Positive results are indicative of active infection with SARS-CoV-2/Flu/RSV; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

37.4 Requirements, Storage and Handling

The applicable Xpert® SARS-COV-2/Flu /RSV (QUAD), SARS-COV-2 (Single), or Flu/RSV Assay kits.

The GeneXpert Instrument and Software.

The Xpert® Assay cartridges and reagents should be stored at 2-28 °C.

- Do not use reagents and cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge and reagents within 30 minutes after opening the cartridge lid.
- Do not use any reagents that have become cloudy.

Proper specimen collection, storage, and transport are critical to the performance of this test.

Inadequate specimen collection, improper specimen handling and/or transport may yield a false result.

Nasopharyngeal, nasal, and mid-turbinate swabs and nasal wash/aspirate specimens can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2–8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems.

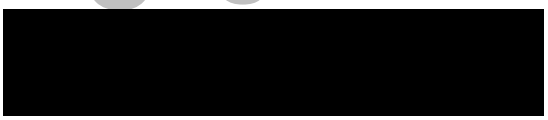
37.5 Procedure

37.5.1 Worksheet

1. Print patient aliquot stickers and place on the GeneXpert Worksheet (MP2.137) and the relevant GeneXpert cartridges to be used.

37.5.2 Instrument Preparation

1. Switch on the GeneXpert first followed by the computer. The reverse order is followed when shutting down the instrument.
2. Log onto the computer at the Windows Log-In Page for Cepheid Admin icon.



3. Click on the GeneXpert DX icon. Enter the following username and password

4. The GeneXpert DX System screen will appear and the computer will connect with the instrument. A self-test will be performed.

5. No further instrumentation preparation steps are required at this stage

37.5.3 Sample Preparation

1. Viral Swabs in VTM - Vortex.

2. Green Top Viral swabs - remove swab and place in a clean VTM. Break swab off so that lid can be applied. Vortex. Alternatively, add 1 ml of sterile water to Green top swab and 'juice' swab. Vortex and pour off into a labelled 2 ml Eppendorf tube.

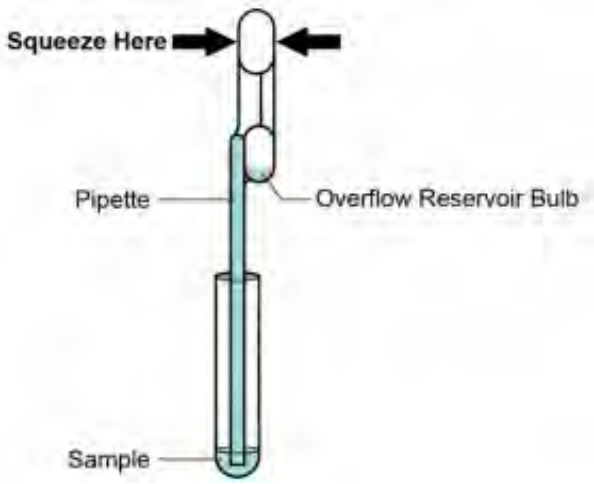
3. Fluids – (NPA's, ETT's, bronchial washings and lavages). Add 600ul of specimen to a clean VTM Vial or 3mLs of sterile saline in a 5ml screw top tube. Vortex.

37.5.4 Preparing the Cartridge

Important: Start the test within 30 minutes of adding the sample to the cartridge.

1. Collect a single Cepheid Xpert® Assay cartridge and Transfer Pipette from the kit located in the Molecular Pathology Section.

2. Place the cartridge, pipette and samples to be tested in a clean biohazard cabinet or workbench. It is not necessary to bring the cartridge to room temperature before use.

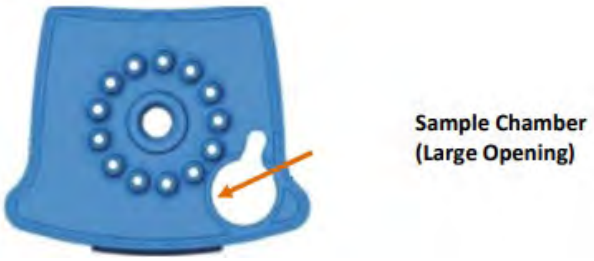


3. In the cabinet or workbench, open the cartridge outer packaging and place the cartridge on the deck. Place a specimen sticker on the side of the cartridge. Check for a small node of plastic left after manufacture on the bottom of the cartridge. Remove the excess plastic node as required.

4. Open the cartridge lid.

5. In the cabinet or workbench, fill the transfer pipette to the *MARK* on the pipette shaft (Figure 1) with the specimen.

6. Using the filled pipette, transfer the entire sample contents to the "S" opening of the Xpert® Assay cartridge (Figure 2). Discard pipette.



7. Close the cartridge lid. Take the cartridge to the GeneXpert instrument. Do not shake or invert the cartridge during transit and place the cartridge on the bench in an upright position.

   The test is invalidated if the cartridge is dropped or shaken prior to testing.

37.5.5 Starting the Test

Important: Before starting the test, ensure the Xpert Xpress SARS-CoV-2/Flu/RSV assay definition files are imported into the software. Assay definition files are on the supplied CD.

1. At the GeneXpert DX System computer screen, select Create Test.

2. In the Patient ID box, enter the patient SURNAME.

3. In the Sample ID box, enter the X Number.

4. Cancel Host query prompts.

5. Scan the cartridge barcode upon request.

6. Select the assigned module to be used. Add further information to Notes as needed. Click Start Test.

7. Open the instrument module door with the blinking green light and load the cartridge. HOLD the door CLOSED until the door is held closed by the instrument. The run will start and run for 30, 50 or 60 minutes (depending on which cartridge is running).

8. When the test is finished, the green light turns off. When the system releases the door lock, open the module door and remove the cartridge. Dispose of the used cartridges in the hazardous waste bin.

37.5.6 Quality Control

Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC) and a Probe Check Control (PCC).

Sample Processing Control (SPC)—Ensures the sample was correctly processed. The SPC verifies that binding and elution of target NA have occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay. The SPC should be positive in an analyte negative sample and can be negative or positive in an analyte positive sample. The SPC passes if it meets the validated acceptance criteria.

Sample Adequacy Control (SAC)—Ensures that the sample contains human cells or human DNA. This multiplex assay includes primers and probes for the detection of a single copy human gene. The SAC signal is only to be considered in an analyte negative sample. A negative SAC indicates that no human cells are present in the sample due to insufficient mixing of the sample or because of an inadequately taken sample.

Probe Check Control (PCC**)—**Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.

An **external Quality Control (**QC) sample must be run on all new lot numbers and deliveries as they arrive into the laboratory and the results valid before the new kits can be used (refer to document MP1.08). Report QC results in LIMS and store records in the GeneXpert QC folder.

Do Not use a kit that does not have a Green 'Q.A passed' sticker on it.

37.5.7 Interpretation of Results

The results are automatically interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.

The Xpert Assays provides test results according to algorithms shown below. The final results for each individual Xpert Xpress cartridge are shown below in Tables 1, 2 and 3.

Table 1. Xpert Xpress SARS-CoV-2_Flu_RSV Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target RNA is detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	• The Flu A signal for either the Flu A1 RNA target or the Flu A2 RNA target or signals for both RNA targets has a Ct within the valid range and endpoint above the threshold setting. • SPC - NA; SPC is ignored because the Flu A target amplification occurred. • Probe Check - PASS; all probe check results pass.
Flu B POSITIVE	• The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because Flu B target amplification occurred. • Probe Check: PASS; all probe check results pass
RSV POSITIVE	• The RSV signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because RSV target amplification occurred. • Probe Check: PASS; all probe check results pass.

Table 1. Xpert Xpress SARS-CoV-2 Flu RSV Possible Results and Interpretation (Continued)

Result	Interpretation
SARS-CoV-2 NEGATIVE; **Flu A NEGATIVE;** **Flu B NEGATIVE;** **RSV NEGATIVE**	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. • SPC - PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and all targets are not detected. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signals do not have a Ct within valid range and endpoint is below minimum setting. • Probe Check - PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV RNA cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: NA
If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.	

Table 2. Xpert Xpress 'Single' SARS-CoV-2 Possible Results and Interpretation

Xpert Xpress SARS-CoV-2

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POS	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested according to the Retest Procedure in Section 17.2. For samples with a repeated presumptive positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. • The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting • SPC: NA; SPC is ignored because a target amplification has occurred. • Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. • The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting • SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting • Probe Check: PASS; all probe check results pass

Xpert Xpress SARS-CoV-2

Result	Interpretation
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting • Probe Check – PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in Section 17.2. A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

The Xpert Xpress SARS-CoV-2 test includes an Early Assay Termination (EAT) function which will provide earlier time to results in high titer specimens. When SARS-CoV-2 titers are high enough to initiate the EAT function, the SPC amplification curve may not be seen and its results may not be reported.

Table 3. All Possible Final Test Results for the Xpert Xpress Flu/RSV.

Result Text	Flu A 1	Flu A 2	Flu B	RSV	SPC
Flu A POSITIVE; Flu B NEGATIVE; RSV NEGATIVE	POS	POS/NEG	NEG	NEG	POS/NEG
	POS/NEG	POS			
Flu A POSITIVE; Flu B POSITIVE; RSV NEGATIVE	POS	POS/NEG	POS	NEG	POS/NEG
	POS/NEG	POS			
Flu A POSITIVE; Flu B NEGATIVE; RSV POSITIVE	POS	POS/NEG	NEG	POS	POS/NEG
	POS/NEG	POS			
Flu A POSITIVE; Flu B POSITIVE; RSV POSITIVE	POS	POS/NEG	POS	POS	POS/NEG
	POS/NEG	POS			
Flu A NEGATIVE; Flu B POSITIVE; RSV NEGATIVE	NEG	NEG	POS	NEG	POS/NEG
Flu A NEGATIVE; Flu B NEGATIVE; RSV POSITIVE	NEG	NEG	NEG	POS	POS/NEG
Flu A NEGATIVE; Flu B POSITIVE; RSV POSITIVE	NEG	NEG	POS	POS	POS/NEG
Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	NEG	NEG	NEG	NEG	POS
INVALID	NEG	NEG	NEG	NEG	NEG
ERROR	NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT
NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT	NO RESULT

37.5.7 Failed Results – Error Messages

In all cases of failed results, repeat the assay according to the procedures described above.

37.5.8 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to instructions in the following section titled “Retest Procedure”.

- An INVALID result indicates that the controls SPC failed. The sample was not properly processed or PCR is inhibited.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.

-
- A NO RESULT indicates that insufficient data was collected. For example, the operator stopped a test that was in progress.

37.6 Reporting Results

37.6.1 Laboratory Information System

All results need to recorded in the Laboratory Information System (LIS). Confirm the results have been auto downloaded into the relevant fields. If this has not occurred, manually enter each result and associated Cycle Threshold (CT). A second scientist must confirm manual entry prior to authorisation.

37.6.2 Authorising Results

The results and data entry must be checked by a senior scientist before release.

The following needs to be checked by the authorising officer.

1. Assay results are deemed acceptable and the data transcription to the worksheet is accurate.

2. The data entry of the result and patient details from the referral are correct.

37.7 Calvary Laboratory Protocol

Calvary Laboratory are now performing Sars-Cov-2 (singles) and Sars-Cov-2/Flu/RSV (quad) Genexpert testing.

37.7.1 Testing Procedure

Print patient aliquot stickers and place in the GeneXpert results Entry Book (CV02.252) and the relevant GeneXpert cartridges to be used (single or quad).

37.7.2 Instrument Preparation

6. Switch on the GeneXpert first followed by the computer. The reverse order is followed when shutting down the instrument.
7. Log onto the computer at the Windows Log-In Page for Cepheid Admin icon.

[redacted]

8. Click on the GeneXpert DX icon. Enter the following username and password

[redacted]

9. The GeneXpert DX System screen will appear and the computer will connect with the instrument. A self-test will be performed.

10. No further instrumentation preparation steps are required at this stage

37.7.3 Sample Preparation

1. Viral Swabs in VTM - Vortex.

2. Green Top Viral swabs - remove swab and place in a clean VTM. Break swab off so that lid can be applied. Vortex. Alternatively, add 1 ml of sterile water to Green top swab and 'juice' swab. Vortex and pour off into a labelled 2 ml Eppendorf tube.

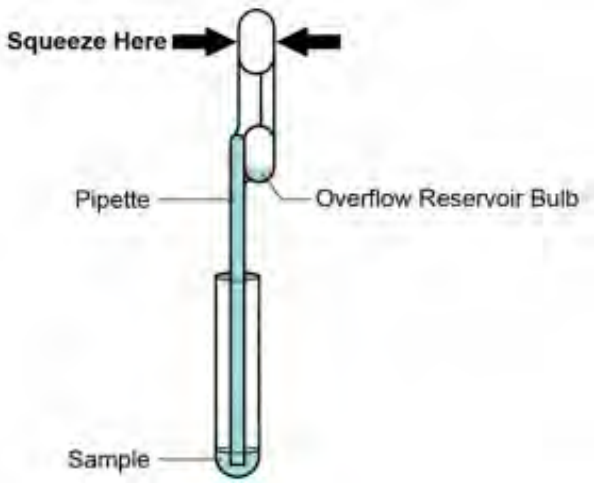
3. Fluids – (NPA's, ETT's, bronchial washings and lavages). Add 600ul of specimen to a clean VTM Vial or 3mLs of sterile saline in a 5ml screw top tube. Vortex.

37.7.4 Preparing the Cartridge

Important: Start the test within 30 minutes of adding the sample to the cartridge.

8. Collect a single Cepheid Xpert® Assay cartridge and Transfer Pipette from the kit located in the Molecular Pathology Section.

9. Place the cartridge, pipette and samples to be tested in a clean biohazard cabinet or workbench. It is not necessary to bring the cartridge to room temperature before use.



10. In the cabinet or workbench, open the cartridge outer packaging and place the cartridge on the deck. Place a specimen sticker on the side of the cartridge. Check for a small node of plastic left after manufacture on the bottom of the cartridge. Remove the excess plastic node as required.

11. Open the cartridge lid.

12. In the cabinet or workbench, fill the transfer pipette to the *MARK* on the pipette shaft (Figure 1) with the specimen.

13. Using the filled pipette, transfer the entire sample contents to the "S" opening of the Xpert® Assay cartridge (Figure 2). Discard pipette.

14. Close the cartridge lid. Take the cartridge to the GeneXpert instrument. Do not shake or invert the cartridge during transit and place the cartridge on the bench in an upright position.

    The test is invalidated if the cartridge is dropped or shaken prior to testing.

37.7.5 Starting the Test

Important: Before starting the test, ensure the Xpert Xpress SARS-CoV-2/Flu/RSV assay definition files are imported into the software. Assay definition files are on the supplied CD.

1. At the GeneXpert DX System computer screen, select Create Test.

2. In the Patient ID box, enter the patient SURNAME.

3. In the Sample ID box, enter the X Number.

4. Cancel Host query prompts.

5. Scan the cartridge barcode upon request.

6. Select the assigned module to be used. Add further information to Notes as needed. Click Start Test.

7. Open the instrument module door with the blinking green light and load the cartridge. HOLD the door CLOSED until the door is held closed by the instrument. The run will start and run for 30, 50 or 60 minutes (depending on which cartridge is running).

8. When the test is finished, the green light turns off. When the system releases the door lock, open the module door and remove the cartridge. Dispose of the used cartridges in the hazardous waste bin.

37.7.6 Failed Results – Error Messages

In all cases of failed results, repeat the assay.

37.7.7 Reasons to Repeat the Assay

If any of the test results mentioned below occur, repeat the test according to instructions in the following section titled “Retest Procedure”.

- An INVALID result indicates that the controls SPC failed. The sample was not properly processed, or PCR is inhibited.
- An ERROR result indicates that the Probe Check control failed, and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded.
-
- A NO RESULT indicates that insufficient data was collected. For example, the operator stopped a test that was in progress.

37.7.8 Interpreting Results

Place **Tested at Calvary** in the silent comments for all COVID tests performed at Calvary. Print a report for all positive results:

To print a report from GeneXpert:

- Click on *view test* icon up the top of the screen, then on the bottom task bar select *view test*
- Highlight the patient you want to send and press Ok
- Select *report*
- Then select *preview PDF*
- Go to file in top left of PDF and select *save as PDF*. Select computer->Store N Go
- Save to the USB.
- Upload file to computer, print
- Scan to LIS episode number and send print out with primary sample to TCH

37.7.8.1 Not Detected results

- All negative results are to be entered into the **GeneXpert results Entry Book (CV02.252)** with ct(s) noted as “0”**.**
- A scientist must then check the results, add a “negative for x targets” in the silent comment field and authorise the result.

37.7.8.2 Detected results (Ct<39.0)

- Enter "*DETECTED*'' into the result field (i.e., Coronavirus SARS-Cov-2, influenza A/B/RSV).

a. **Note** that for Sars-Cov-2 (singles), both targets must be present with ct <39 to report as *"DETECTED"*. For Sars-Cov-2 (quads) the target must be present with a ct <39 to report as *"DETECTED"*.
b. **Note** that for influenza A, if either one of Flu A1 or FluA2 has Ct ≤39.0 or if both have Ct ≤39.0, then "*DETECTED*" can be entered into result field.

- Transcribe the 'Ct value' into the 'GX-E' and 'GX-N2' field for SARS-CoV2 (singles) OR 'GX-FLU' field for influenza (Flu A1 and A2 must both have a Ct value) or 'GX-RSV' for RSV (quads).
- Proceed to interim/verify the report and inform the ward of the result via phone.

37.7.8.3 Indeterminate results (Ct(s)≥39.0)

Note that the analyser will call these "Detected" for all tests.

- For **Sars-Cov-2** (singles):
  - if SARS CoV-2 is detected on instrument with both **Ct values greater than 39.0** OR if **only one** **Ct value is detected and greater than 35.0**
    - enter as ***'INDETERMINATE'*** (indeterminate comment is autogenerated).
    - Place into interim verify so that clinicians can see interim result on computer and phone to ward.

- For **Sars-Cov-2/Flu/RSV** (quads):

  - If **Sars-Cov-2** is detected on instrument but **Ct Value is greater than 39.0**
    - enter result as '***INDETERMINATE'*** (indeterminate comment is autogenerated).
    - notify requesting ward result is '*Indeterminate*'.

  - If **Influenza A** is detected on instrument on **either one (A1 or A2)** of the Influenza A targets **but not on both** the targets, and the one detected target has **Ct Value is greater than 39.0,** OR if Influenza A is detected on instrument **on both** Influenza A targets but **both targets Ct Value is greater than 39.0** then:
    - enter result as "*Indeterminate*"– comment is autogenerated
    - notify requesting ward result is '*Indeterminate*'.

  - If **Influenza B** is detected on instrument but **Ct Value is greater than 39.**
    - enter result as "Indeterminate"– comment is autogenerated
    - notify requesting ward result is '*Indeterminate*'.

  - If **RSV** is detected on instrument but **Ct Value is greater than 39.0**
    - enter result as "Indeterminate"– comment is autogenerated
    - notify requesting ward result is '*Indeterminate*'.

37.8 Method Limitations

Performance of the Xpert Xpress SARS-CoV-2/Flu/RSV, SARS-CoV-2, and Flu/RSV test have only been established in nasopharyngeal swab and nasal wash/aspirate specimens. Use of the Xpert Xpress SARS-CoV-2/Flu/RSV, SARS-CoV-2, and Flu/RSV test with other specimen types has not been assessed and performance characteristics are unknown.

A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.

As with any molecular test, mutations within the target regions of SARS-CoV-2, Flu (Flu A1, A2 and B) or RSV could affect primer and/or probe binding resulting in failure to detect the presence of virus.

This test cannot rule out diseases caused by other bacterial or viral pathogens.

The Xpert SARS-CoV-2/Flu/RSV, SARS-CoV-2, and Flu/RSV Assays provides qualitative results only.

37.9 Associated Documentation

MP1.08 – QC and QA Specimens and Procedures
MP2.137 GeneXpert SARS-CoV-2 & Flu A/B/RSV Worksheet
MP9.56 Xpert Xpress Flu RSV
MP9.62 Xpert Xpress SARS-COV-2
MP9.63 Xpert Xpress SARS-COV-2/Flu (Flu A1, A2 and B)

Calvary Laboratory

DG12.09 GeneXpert System Maintenance
CV03.411 GeneExpert Results
CV02.252 PCR Testing Worksheet

48. SeeGene Allplex SARS-COV-2/FLUA/FLUB/RSV Assay

48.1 Test Mnemonic

SeeGene Allplex SARS-COV-2/FLUA/FLUB/RSV Assay.

48.2 Purpose

Allplex™ SARS-CoV-2/Flu A/Flu B/RSV Assay is in vitro diagnostic medical device designed for qualitative detection of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) with real-time reverse transcription PCR from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.

Multiplex real-time one-step RT-PCR system for detection of SARS-CoV-2, Influenza A virus, Influenza B virus, and Human respiratory syncytial virus from nasopharyngeal aspirate, nasopharyngeal swab, bronchoalveolar lavage, oropharyngeal (throat) swab, and sputum.

48.3 Principle

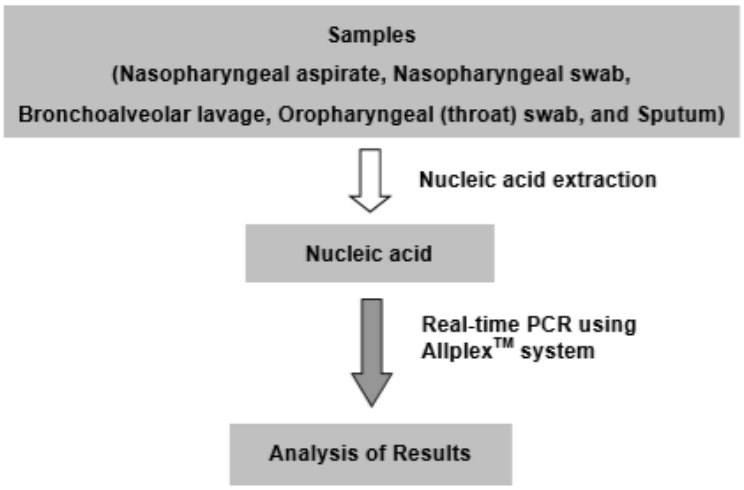
Allplex™ SARS-CoV-2/Flu A/Flu B/RSV Assay is a multiplex real-time PCR assay that enables simultaneous amplification and differentiation of target nucleic acids of S gene, RdRP gene, and N gene of SARS-CoV-2, Influenza A virus (Flu A), Influenza B virus (Flu B) and Human respiratory syncytial virus (RSV) with two Internal Controls (Endogenous IC and Exogenous IC). To perform the multiplex target amplification and detection with superior accuracy in a single reaction, this assay kit employs Seegene's innovative proprietary DPOTM, TOCETM and MuDTTM technologies. The presence of specific gene sequences in the reaction is reported as a Ct value through Seegene Viewer analysis software.

Please note: Both endogenous gene and exogenous gene are used as Internal Control (IC).

Endogenous IC is used to check the proper sample collection.

Exogenous IC is used to monitor the whole process from nucleic acid extraction and to check for any possible PCR inhibition.

Two ICs enable to demonstrate proper sample collection and test validity of each specimen in a single reaction. To prevent amplification product from acting as potential contaminants, Uracil-DNA glycosylase (UDG)-dUTP system is employed in Allplex™ SARS-CoV-2/Flu A/Flu B/RSV Assay. The UDGdUTP system is commonly used when performing PCR to eliminate amplicon carry-over using UDG to excise uracil residues from DNA by cleaving the N-glycosylic bond.



< Allplex™ SARS-CoV-2/Flu A/Flu B/RSV Assay procedure overview >

1. severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Coronavirus is a RNA virus which contains approximately 27~32 kb of positive single stranded RNA genome. In human, it causes respiratory tract infections that can range from mild to lethal. In December 2019, a number of patients with pneumonia of unknown aetiology emerged in Wuhan, China and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus has been identified as the causative agent. Coronavirus disease 19 (COVID-19) has been spreading around the world and the World Health Organization (WHO) has declared COVID-19 pandemic in March 2020. By August 2020, more than 20,000,000 confirmed cases and more than 700,000 deaths of COVID-19 have been reported to the WHO worldwide. SARS-CoV-2 is transmitted through droplets from infected patients. The COVID-19 infected patients have clinical manifestation which includes the fever and cough as primary clinical presentations and others are shortness of breath and myalgia etc. However, asymptomatic infection cases are also emerging, so it is very important to quickly diagnose and take quarantine measures.

2. Influenza Virus

Influenza virus (family Orthomyxoviridae) is an enveloped virus with a single, negative strand RNA genome that is present in 8 separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses, and it may contribute to a rapid development of new influenza strains through the interchange of gene segments between different viruses infecting the same cell. There are three types of influenza viruses: A, B, and C. A and B viruses are most common among the three. Especially type A virus is known to be common for infecting not only humans, but also birds and various types of mammals. Among the influenza viruses that invade humans, H1N1 and H3N2 are becoming a problem for infecting children and elder seniors.

3. Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (family Paramyxoviridae) is an enveloped virus with a single, negative strand RNA genome. RSV has a high frequency of occurrence among other respiratory diseases. It is categorized as clinically significant for being able to cause death in babies with congenital malformation of cardiac, premature infants, and babies right after open heart surgery. Bronchiolitis is the most common type of RSV infection in infants, but does not appear as often after one year old. On the other hand, pneumonia is a problem for all infants. RSV is responsible for developing 45~75% of bronchiolitis, 15~25% of infant pneumonia, and 6~8% of croup. RSV was originally known as pathogens developing diseases occurring in babies, but recent studies show that RSV occurring in adults can produce results as severe as influenza such as morbidity and leading to deaths. In addition, if patients with declined immune systems (who have gone through bone-marrow transplantation or solid organ transplantation) are infected with RSV that causes pneumonia, the probability of death can rise up to more than 50%.

The reagents in one kit contains 100 tests.

Order information: (Cat. No. RV10259X)

All components of Allplex™ SARS-CoV-2/Flu A/Flu B/ RSV Assay should be stored at ≤ -20°C.

All components are stable under recommended storage conditions until the expiry date stated on the label.

The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay			
Symbol	**Contents**	**Volume**	**Description**
PRIMER	SC2FabR MOM	500 µL	Oligo Mix : - Amplification and detection reagent
PREMIX	EM8	500 µL	- RTase - DNA polymerase - Uracil-DNA glycosylase (UDG) - Buffer containing dNTPs
CONTROL +	SC2FabR PC	100 µL	Positive Control (PC): - Mixture of pathogen and IC clones
CONTROL IC	RP-V IC 2	1,000 µL	Exogenous Internal Control (IC)
WATER	RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade
	User manual		

** Bronchoalveolar lavage has not been validated with the MagNA Pure 96.

48.4 Nucleic Acid Extraction

Follow procedure MP5.04. Add internal control to the specimen as detailed below.

Internal Control

The internal control included in the kit allows the user not only to verify the nucleic acid extraction procedure but also to identify any PCR inhibition.

Using the Hamilton microlab star place two tubes of RP-V IC 2 to aliquot 10uL into the extraction block (processing cartridge) on the MP96. Refer to document MP4.47 for full instructions for the Hamilton Microlab Star aliquoting robot.

If extracting on the chemagic extractor, the internal control is added into the lysate cocktail.

If extracting on the MT-PREP manually aliquot 10uL into the sample tube.

If Hamilton is offline using an ovation pipette aliquot 10uL of IC for all patient wells including wells 93 & 94 (controls).

48.5 Calibration

A **Positive Control QC** sample is supplied by the kit and must be included in every run.
A **PCR negative water QC** sample from the kit is included in every run.
An **extraction negative QC** sample from the extraction is included in every run.
A **Positive SARS-COV-2/FLUA/FLUB/RSV extraction QC** sample is included in every extraction and test run.

48.6 Calibrator Traceability

Crossing point values for positive QC samples are recorded in LIMS and reviewed every six months.

Interfacing information for set up to follow********INTERFACING HAS NOT YET BEEN SET UP.**

48.7 Extraction on the Roche MP96 Extraction

A. Computer Setup:

- Open the MP96 software on the desktop. Log on – [redacted]
- The MP96 extractor is cleaned after use, so cleaning of the instrument should already be done.

- Select Instrument tab>Click on Daily maintenance (Five ticks will appear on MP96 - Maintenance, Daily maintenance, Prime, confirm clean instrument & confirm Daily Maintenance)>Click Start to begin (Priming with system fluid will complete in less than a minute and daily maintenance is complete.

- Select the **Overview** tab, then the **Enter Order** icon. On the left side, click the dropdown menu under **MP96 Kit Name.** Select the "**DNA/Viral NA SV 2.0**" kit. click the dropdown menu under **Protocol**, select the "**Viral NA Universe SV 4.0**" Kit. Select the sample volume as **200uL** and the elution volume as **50uL**.

- Now enter the sample names on the sheet: For a cartridge prepare on the Hamilton robot, a template can be used (such as "-" for all fields). Change sample number 1 to the first pool number (pool number stuck on the MP96 cartridge/Cover sheet). Enter "POS Ext" on well 93 and "Neg Ext" on well 94. Wells 95 and 96 stay blank.

- Now save the run (top right save icon) with a "***PLXxxxx yyyymmdd hhmm***" format. The machine can now be set up for the run saved. Select the arrow on the bottom right after saving to proceed to the layout of the deck.

B. MP96 Setup (*A full run will need a kit with at least 96 tests left*):

- Place the set of magnetic beads in the first, narrower, drawer in the first two slots, if two kits with same lot is used place the second set in the second location.
- Place x2 empty cartridges, in the top slot of the second and third drawer.
- Place the MP96 kit reagent cassettes (Tray 1 and 2) in one of these drawers, (second or third)
- Ensure there are enough tips in the fourth drawer along for a full run – at least two full tip racks of 96 tips.
- Place an MP96 output plate in the fifth drawer, at the bottom front (stainless steel 96 plate section).
- Ensure the sixth drawer is free of used tips – empty tip racks should replace the full used racks (plenty of them in the cupboard next to the machine).
- Close the machine so it can initialise before adding the cartridge with samples in it from the Hamilton.
- Once the Hamilton has completed its run, manually assess the liquid levels in the processing cartridge. Check if an excluded file has been created, if not, then manually aliquot 200uL of correct primary specimen to respective wells that appear low in volume (use CFX file to confirm correct transfer).
- Add header and borders and comments section to the LIMS FILE and perform conditional formatting for duplicate X numbers/P or N numbers.
- Print out the LIMS file from the appropriate folder, if any address any excluded samples from the excluded file located on the desktop (middle section) and sign that you have addressed any empty or low-level wells.

- On the MP96 Carefully place the prepared cartridge in the top first slot of the fifth drawer. Please ensure correct orientation with barcode (i.e.: A1 is top left).
- Close the drawer and the MP96 load flap. Allow machine to initialise.
- On the computer software, the layout of the deck can be assessed as the machine initialises. The fields should turn green as they are initialised.
- When the machine has finished initialising it will loudly "click" and the "start run" icon on the top right of the computer screen will become green. Press this button to start the run. The progress screen will show how far along the run is as it progresses.
- One run takes ~ 60 minutes.
- When the run has completed, Open the bottom flap, remove the output plate (consists of extracted nucleic acid)
- The results of the run can be checked as indicated on the screen "Check Last Results". The whole plate should be green if extraction was successful.
- If the extraction "failed" create a problem report and contact Roche and wait for further instructions.

C. Prepare the real-time PCR Mastermix

Note: The Bio-Rad CFX-96 plates and caps must be used. Aerosol resistant filter tips and tight gloves must be used when preparing One-step RTPCR reactions. Use extreme care to prevent cross-contamination. Completely thaw all reagents before use. Keep cool once thawed. Briefly centrifuge reagent tubes to collect residual drops inside of the cap.

5 µL	SC2FabR MOM
5 µL	EM8
10 µL	Total volume of Mastermix

10 µL	Reaction Mastermix
10 µL	Sample's nucleic acid
20 µL	Total volume of reaction

- ***Please Note: For this assay half or part of the kit may need to be used if you have <92 patients to test. If this is the case, you will need to write down the number of reactions used and number of reactions left on the kit and make sure you do not discard any of the reagents in the kit for use for the next run.***

- ***If half/part of run is processed the controls (positive and negative) are always in position 93 & 94 on the Hamilton. To avoid extracting a full MP96 plate when there is <92 patients, the controls will have to be moved from positions 93 & 94 to the new positions on the MP96 extractor and amended on the CFX file to reflect this.***

- ***The kit components are not affected for up to 5 freeze/thaw processes. If the reagents are to be used only intermittently, they should be stored in aliquots.***

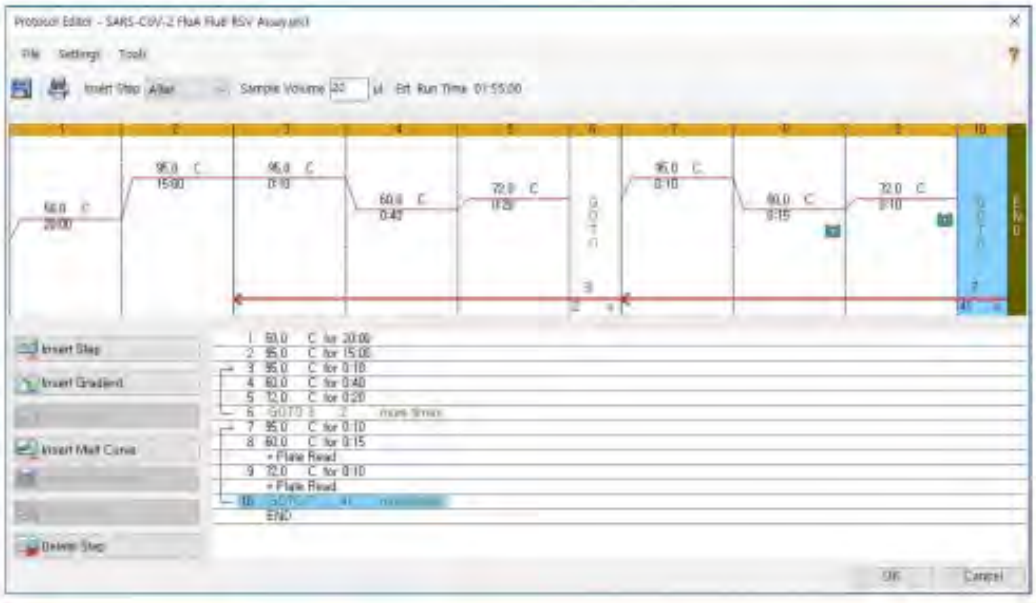
Note: Calculate the total amount of each reagent needed based on the number of reactions including samples and controls. One full run of 96 samples will require 540uL (x108 rxns) of each of the two reagents (i.e., n+ 1 for every column of 8 samples). If it is a run of 48 you will need to calculate n+ 6, thus require 270uL (x54rxns) of each of the two reagents.

- Take the Seegene mastermix out of the freezer when MP96 run is at 50% and leave in the cabinet to thaw in the clean room. Place the IC (orange lid) in the green box in the fridge (labelled SARS-FLU-RSV ASSAY reagents).
- Place the Kit Positive (green lid) out into the lab to thaw.
- Prepare the Mastermix in the clean room in an amber tube aliquot 540uL each of the SC2FabR MOM and EM8. Mix by inversion and quick spin.
- Take out a Seegene 96 well plate. Using the electronic pipette, pipette 10uL into each well as required using the "MULTI" program. Program this to "10uL MULTI" program to dispense 24 wells of master mix. Change the pipette every third row.
- The "MULTI" program can easily be changed to "15uL MULTI" when setting up for the Sars-Cov-2 only assay.
- Once the plate has been pipetted, cover the plate (kimwipe). Take the plate and remaining mastermix (in the amber tube) out into the DNA lab PCR setup corner.
- Place the plate and mastermix tube on a freezer brick if PCR setup is delayed (in bottom of -20C freezer).
- Using the multi-channel pipette and yellow rack small pipette tips, select the program "**CORFLURSV_SEEGN",** which pipettes 10uL in 8 channels.
- Pipette first 11 columns from the MP96 output plate into the Seegene plate. **Be careful that you don't pipette from the Seegene plate into the MP96 output plate and ensure the correct rows are being pipetted.** You can place a cover over the rows to be pipetted to show which rows have already been pipetted.
- For **column 12** pipette the SIX samples using the multi-channel pipette and then **Pipette 10uL of RNAse Free Water into well 95, and 10uL SC2FabR PC (Kit POS) into well 96 using the single 1-10uL pipette**.
- Once all samples have been pipetted, place the 8-strip plastic cap covers on.
- Spin the plate in the centrifuge located in the agar/media room. Or in the spinner near EP2/EP3 (Make sure the bottom of the plate is facing outwards).

D. Thermal Profile

Step	No. of cycles	Temperature	Duration
1	1	50°C	20 min
2		95°C	15 min
3	3	95°C	10 sec
4		60°C	40 sec
5		72°C	20 sec
6	GOTO Step 3, 2 more times		
7	42	95°C	10 sec
8*		60°C	15 sec
9*		72°C	10 sec
10	GOTO Step 7, 41 more times		

Note*: Plate Read Step. Fluorescence is detected at 60°C and 72°C (Step 8 and 9).



E. Thermocycler Run Setup and CFX/LIMS data files

- IF switched off, switch ON the CFX96 Thermocycler and then Computer.
- Press the Hatch button on the CFX96 Thermocycler to open the Hatch. Wait for the Hatch to open completely and load the PCR Plate into one of the Thermocyclers (Atom/Jerry).
- Press the Return Hatch button to close the hatch.
- Select the **SARSCOV2_FluA_FluB_RSV Protocol** on the desktop (bottom – right hand side of screen). Press OK on the protocol layout page.
- At the Protocol Editor Window, Select OK. The run conditions have all been pre-loaded.

- At the "Save Optical Data File Window" select the respective "Day Directory" in C:\Desktop\Experiments\YYYY SARSCOV2-FLUA-B-RSV\YYYYMM xxx\"DD" and Save the file under the given name of "admin_YYYY-MM-DD_HH-MM-SS_" Instrument Name". The file is a PCR Data File (*. pcrd). Press SAVE and the thermocycling run starts.
- Select the **Real Time Status** tab, then the **View/Edit plate** option. Then select **Tools → Spreadsheet View** → Click the number column twice to orientate the plate correctly i.e., A1, B1, C1.
- Import the **CORRECT** CFX file for the run from the **Hamilton P** drive (If Hamilton P drive has a red cross, select documents, and open/run map_p_drive.cmd and run program.
- On this window, scroll to the bottom of the page and name the last two blank wells: NEG PCR in well G12 and KIT POS in well H12.
- Check the accuracy of all pool numbers imported. Press "**Exit Spreadsheet View**"
- Set the positive and negative controls by selecting the appropriate well and designating the controls from the first dropdown menu to the right and then press ok:
    - Pos Ext = Positive Control
    - Neg PCR = Negative Control
    - Kit QC = Positive Control

  (Note that the Neg Ext is run as a normal sample, it is not a negative pcr control).
- Once all data is entered correctly, Select OK and Apply Changes "Yes" at the query window.
- Allow the run to complete (~ 1 hour and 43 minutes).
- At this stage the CFX run file that is running can be placed in the archive folder so that the only CFX run files that are yet to be processed are available in P drive.

F. Reviewing and Releasing Results

- Once the run has completed, the "Data Analysis" window will be visible with the raw run data (*.pcrd file).
- Select "Tools" and then "SeeGene Export".
- At the "Browse for Folder" window, the new export file must be saved in the corresponding "Day Directory" at **C:\Desktop\Experiments\ SARSCOV2-FLUA-B-RSV\YYYY xxx\"DD".** Exporting creates two new folders called QuantStep8 and QuantStep9. These are required for analysis by the Viewer software. Export all runs of the day to the same "Day Directory". Do not export directly to the QuantStep8 or QuantStep9 directories.
- Close the "Data Analysis" window and BIORAD CFX Manager software if appropriate.
- Select "SAVE" to save changes to the (*. pcrd) file to the corresponding "Day Directory".
- Open the "Seegene Viewer for Real time Instruments V3" (Red Icon).
- **On PRODUCT tab select Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay.**

- In the Viewer, go to "FILE" then "OPEN" and select the appropriate run (from **C:\Desktop\Experiments\YYYY SARSCOV2-FLUA-B-RSV \ xxx\DD** [Run File.xlsx].

- Run data is shown in the window. Check POS and NEG QC samples are valid. To check validity, the PCR run PC (Positive Control) and NC (Negative Control) are valid when the following criteria are met:

Control	Seegene Viewer Result								
	FAM (C_t)		HEX (C_t)		Cal Red 610 (C_t)		Quasar670 (C_t)		Auto Interpretation
	S gene	RSV	RdRP gene	Flu B	N gene	Flu A	Endo IC	Exo IC	
SC2FabR Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control(+)
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative Control(-)

- Invalid Assay Run
  In case of a validity failure, the results should not be interpreted or reported. And the PCR reaction must be repeated.

Please note: The neg extraction which contains no genomic material will show as invalid due to Endogenous (Endo) IC not amplifying. The negative pcr will also have no amplification for Endo IC but will be valid as there should be no amplification in all four fluorophores. Endogenous IC is used to check the proper sample collection.

Exogenous IC should amplify for all specimens in the run, the only exception being the negative control (Negative PCR) as that is the negative water template. The exogenous IC monitors the whole process from nucleic acid extraction and to check for any possible PCR inhibition.

Result Interpretation

Results are interpreted by the software according to the following table.

1. Analyte Information

Fluorophores	Analytes	
	Graph 1	Graph 2
FAM	S gene	RSV
HEX	RdRP gene	Flu B
Cal Red 610	N gene	Flu A
Quasar 670	Endo IC	Exo IC

2. Interpretation of Results

Analytes	C_t value	Result
Targets	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)
IC	≤ 40	Detected (+)
	> 40 or N/A	Not detected (-)

2-1. Interpretation of SARS-CoV-2

S gene	RdRP gene	N gene	Interpretation of SARS-CoV-2
+	+	+	SARS-CoV-2 RNA, Detected
+	+	-	
+	-	+	
-	+	+	
+	-	-	
-	+	-	
-	-	+	
-	-	-	SARS-CoV-2 RNA, Not detected

2-2. Overall Interpretation

Target Result	Endo IC Result	Exo IC Result	Overall Interpretation
+	+	+	Target Nucleic acid, Detected
	-	+	
	+	-	
-	+	+	Target Nucleic acid, Not detected
	-	+	Invalid [1]
	+	-	Invalid [2]
+/-	-	-	Invalid [3]

1) Sample collection could be incorrect. Repeat from the sample collection. See TROUBLESHOOTINGS section for the detailed instruction.

2) Extraction or PCR could be inhibited. Repeat from the nucleic acid extraction. See TROUBLESHOOTINGS section for the detailed instruction.

3) Repeat from the nucleic acid extraction. If the same result is shown, repeat from the sample collection. See TROUBLESHOOTINGS section for the detailed instruction.

TROUBLESHOOTING

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No signal	The fluorophores for data analysis do not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or expiration of the test kit	Please check the storage conditions (See page 8) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Nucleic acid extraction failure	If IC had been exogenously added to the specimen prior to extraction, the absence of IC signal may indicate loss of nucleic acids during the extraction. Make sure to use recommended extraction method. If due to inhibitors, re-extract the original specimen or the specimen may be diluted with saline buffer 1/3~1/10 fold and then add "RP-V IC2" to the diluted specimen.
No Exogenous Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not Exo IC, then Exo IC amplification may have been inhibited by high titer of target pathogen. If you want to observe Exo IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from RT-PCR step. If the same result is shown, the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.

Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay		
OBSERVATION	**PROBABLE CAUSES**	**SOLUTION**
No Endogenous Internal Control signal	High load of pathogen's nucleic acid	If target pathogen signal is observed but not Endo IC, then Endo IC amplification may have been inhibited by high titer of target pathogen. If you want to observe Endo IC signal, dilute the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Presence of PCR Inhibitor	Please dilute the extracted nucleic acid (1/2~1/5) in RNase-free water and repeat the test from RT-PCR step. If the same result is shown, the specimen (1/3~1/10) in saline buffer and repeat the test from extraction step.
	Incorrect specimen collection	Please collect the specimen again and repeat the test from extraction step.
Putative false positive or target signal(s) observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Only use filter tips throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.

- To carry out conditional formatting and print a copy of the results, on analysed run, tick ALL samples, select “File” then “Export”, and save on a USB stick. Remove USB and take to a computer with printing capability. Open the file in Excel:
  - Change page layout to ‘landscape’ then select margins and then select narrow.
  - Delete the [Sample], [Patient], [Well] [Type] and [Comment] columns.
  - Add the first two columns of LIMS file (cut & paste) to the Seegene results.
  - Insert x2 new columns and then copy and paste POOL & X-number column into columns A & B (start position **A2**) of Seegene results. **(Make sure that the pool numbers align)**.
  - Highlight the relevant cells for printing
  - Select “Page Layout” → Print Area → Set Print Area
  - Select “Print Tiles” → Header/Footer → Custom Header → Insert File Name (icon with green box) → OK.

- On the Page set up tab, "Page" (select drop down arrow in right hand corner) → Fit to 1 page wide and [blank] tall→OK
- To increase font size on print out, reduce column widths.
- If not already done, format borders to add borders to cells → "All Borders"
- Print Paperwork, to print in colour print using follow me and in printer properties select colour and staple to coversheet and LIMS worksheet.
- Use this modified printed results worksheet to enter results in LIMS.

Reporting

- After determining the validity of run (checking that all controls have passed and both IC Ct values are in the mid 20's).
- Interfacing has not been set up. Do not send results to HL7.
- Note any low positives (Flu A/B/Rsv) with Ct values of 39-41 report as Indeterminate.
- For Sars-Cov-2 if a sample has less than 3 of the targets amplify (S gene/RdRP/N gene) sample needs to be repeated on the two other platforms to confirm.
- Enter results manually in CORPCR test code, second check is done by the second scientist who can authorise negatives and interim-verify positives.
- For any requests that have requested full/extended panel add the **Resno** comment in the second field (not where the automated sars-cov-2 comment drops in).
- If working alone, manually enter results and then authorise the negatives and interim-verify positives after some time.

48.8 Internal Quality Control

Manufacturer kit QC and in-house QC are included in each run and crossing points are recorded in LIMS. Outliers are reviewed at the time and all data are reviewed half-yearly.

48.9 EQAP

- The assay is controlled by participation in the RCPA SARS-CoV-2 – 4 Surveys
- RCPA Rapid Influenza – 3 Surveys

48.10 References

- Instructions for use: Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay Cat. No. RV10259X Version 1.0
- MP1.08 QC & QA Specimens & Procedures.

36. Ausdiagnostics FLU RSV SARS-COV-2 Assay

36.1 Test Mnemonic

RDL = DVIRAL
Test Codes = CORPCR

36.2 Purpose

The Ausdiagnostics SARS-CoV-2, Influenza and RSV 8-well Test Kit (Ref#20081 version 8) is a semi-automated qualitative IVD test for the detection of SARS-CoV-2, Influenza and RSV.

36.3 Principle

This assay is used on nucleic acid extracts from swabs, sputum and potentially other specimens.

The assay employs a real-time PCR based method called Multiplexed Tandem Polymerase Chain Reaction (MTPCR) on the High-Plex 24 System. See Instructions For Use - SARS-CoV-2, Influenza and RSV 8-well Test Kit (Ref#20081) for the High-plex 24 system for more details.

36.4 Patient Preparation

No patient preparation is required for sampling for this test.

Appropriate specimen types include swabs (dry, viral and VTM). Sites for swab collection include nasal and throat.

36.5 Requirements

The following is required to conduct this test.

- Ausdiagnostics Hi Plex 24 instrument.
- Step 1 tube micro-centrifuge,
- Robot Tips (Axygen),
- MT-Processor (automates the assay set-up and Step 1 PCR),
- Sealing films for 384-well plate,
- Plate Spinner Centrifuge (Axygen).

The test kits include plates and strip tubes that are stored at room temperature in the radiation room. **Demi RNA Master Mix cassettes** are stored in the PCR setup lab (Clean room) in the -20ºC freezer.

36.6 Calibration

Yearly maintenance is performed on the instrument by the manufacturer to maintain correct instrument parameters.

36.7 Calibrator Traceability

None.

36.8 Procedure

36.8.1 Check in Specimens

Check that specimen details (ie. patient name, DOB, UR number, test sets) on the specimen and request form match the patient record in LIMS.

To check the above details in LIMS, perform the following steps:

1. Select Services in the Patient Data drop-down, enter & select Aliquots press enter and select Molecular Aliquot TLP2824 press [X] for execute.
2. Scan the specimen barcode to open the patient data record screen, press D to go to the main screen. Press ALT-D to open the request form. Check all data entry matches the specimen & the form and that all test codes have been entered correctly.
3. Press Esc three times and the space bar to print out X numbers, stick one X number on the primary specimen, another one on the Viral Worksheet, and the remaining X numbers are stuck on the 1.5ml elution tube and 1.5 ml flip top tube (required for swab specimens only).
4. All specimens that are checked in should have X numbers stuck on the worksheet in the order in which they arrive in the laboratory. On the Viral Worksheet the two repeat spots are not to have labels stuck on in case there are repeat extractions that will be required for the next run.
5. After all specimens have been checked in and X number labels have been stuck on the worksheet, specimens are extracted on the automated MP96 extractor.

36.8.2 Sample Preparation

Extract 200ul of swab sample on the automated MP96 extractor using the universal pathogen protocol with cRNA and elute into 50ul. Extracted specimens are to be processed within 30 minutes of extraction or stored in the -20^{0}C freezer until testing. (See MP05.11)

Transfer nucleic acid extracts from output plate to elute tubes using the Qiagility (See MP10.03).

36.8.3 Worklist

Create a Worksheet from the Laboratory Information System. Select Patient Data, Services, Molecular Worklists, Worksheet. Select X and P to print worksheet.

Each assay must include a Positive Control (Respiratory pathogens REF 91011.07), RSV positive control and a negative extraction control.

36.8.4 Preparation of the High-Plex platform

1. During extraction of specimens prepare 4 mL of bleach containing 0.4% available chlorine to a 5 mL tube for each platform.

2. Collect **Demi RNA Master Mix cassette** from the clean room freezer and allow to thaw for at least 30 minutes in a dark cupboard drawer or in a light proof canister in the DNA extraction lab. (Once the Master mix is thawed, it cannot be re-frozen).

3. Organise your worklist and number your specimens and controls accordingly. Remove the appropriate number of cassettes (containing Step 1and Step 2 master-mix, water, and oil) from the freezer in the clean room (one cassette required for each set of 8 samples or part thereof (see Figure 1). Write down all required lot numbers and expiry dates on worksheet.

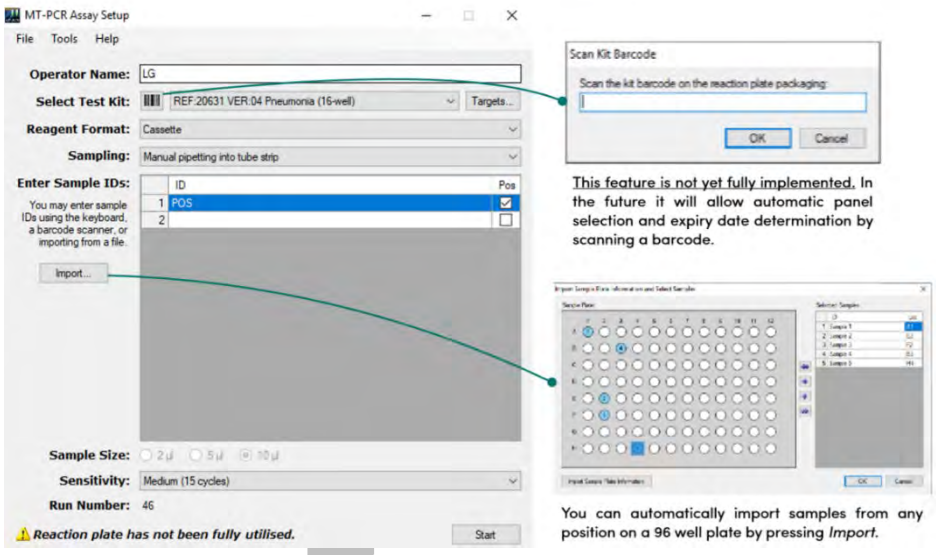
4. Take your worksheet and 0.4% bleach to the Hi-Plex platform & ensure a plastic waste bag is in place over the waste chute.

5. Switch on the MT-Processor, MT-Analyser and computer software in that order, and the reverse when shutting down platform.

6. Open the MT-Plex™ Assay Setup software icon on the desktop

7. Add the following information to the MT-Plex™ Assay Setup: Operator’s name

Select: REF 20081P VER08 SARS-CoV-2, Influenza and RSV 8-well from the drop-down menu.



Select which reagent format you are using: i.e reagent cassettes.
Type/Scan X numbers of controls and specimens to be tested from the Viral Worksheet into Enter Sample IDs (alphanumeric only, no special characters) Note: If you want to auto-sample the positive controls from the reagent cassette block, you must tick the 'Pos' check box next to the sample ID.

Note if the Kit Template is not visible, the template file (.MT file) needs installing. See Appendix. It is very important that the selected file number is identical to the catalogue and version number of the kit (found on the label on the plate kit).

1. The **STEP 1 TUBES** pack must have matching catalogue number and version number to the **STEP 2 PLATE** label.

2. Mark the tube strips with the correct orientation, stating the first position. If you have more than one tube strips, mark with different identifiers (i.e U1, U2, & U3)

3. Using the QIAgility when extraction is complete on the automated extractor, transfer extracted nucleic acid from the output plate of the MP96 extractor to the respective elution tubes.

4. Whilst transfer of extracted nucleic acid from the output plate is being processed by the QIAgility, the foil seals on the STEP 1 TUBES can be pierced with the piercing tool provided by AusDiagnostics or a clean pipette tip or.

Note: It is recommended to clean the AusDiagnostics piercing tool regularly by soaking in bleach with 0.4% available chlorine for 10 minutes.

5. Using an electronic multichannel pipette transfer 10uL of extracted nucleic acid into the bottom of the corresponding Step 1 tube strip wells.

6. In the Radiation room place the Step 1 strip tubes in a quick spin centrifuge and pulse spin to collect the sample into the base of the wells. Place the tube strip containing sample in the MT-Processor and close the cover. The first sample should always be in the top left of the cycler (Figure 1) and subsequent sample strips should be placed consecutively to the right of the previous strip. Cover the thermal cycler block with the black plastic cover.

7. Thawed cassette reagents are mixed by inverting five times then spun at low g force for 5 seconds to remove any liquid from the lid.

8. Place the required reagent cassettes in the Reagent Block of the robot as shown in Figure 1.

Things to check just before running the instrument.

- 96-well Dilution Plate fitted as per Figure 1 (it can be orientated in either direction)
- Step 2 plate (High-Plex plate) fitted as per Figure 1 *(NB: Unpack the Step 2 plate from pouch & fit on instrument just before running the assay as the plate contains dried oligonucleotides and light sensitive fluorescent probes).*
- Spun reagent cassettes in the reagent block and the reagent block lid is closed.
- Tips match tip availability in screen tip image (make sure the tip racks are sitting flush). Check Tip Availability in the software (described below)
- Uncapped bleach is in place.

7.2 SETUP PROCESSOR DECK

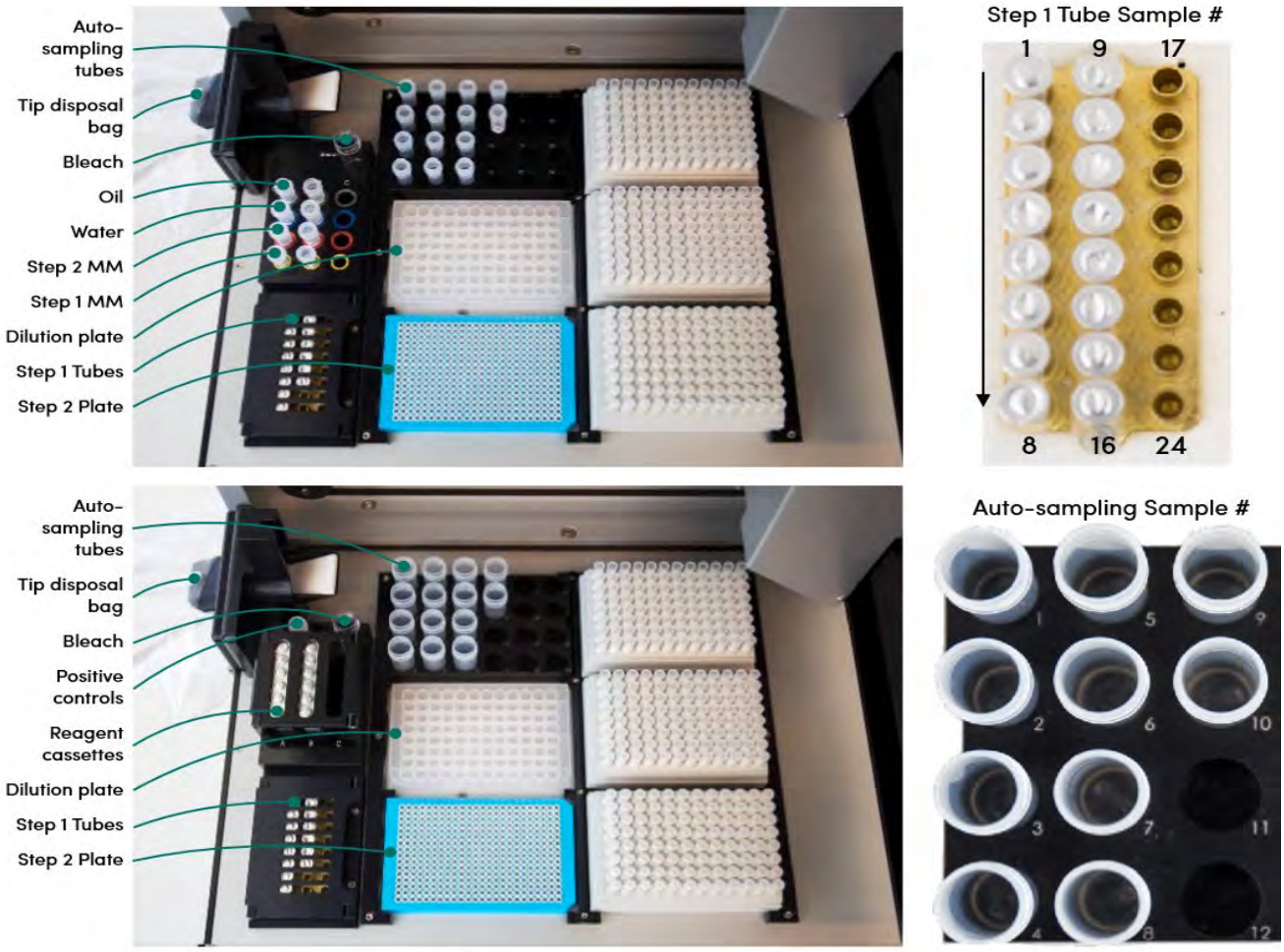


Figure 4. High-Plex 24 deck and sample layout.

IMPORTANT: The run must be started within 30 minutes of thawing the Mastermix.

The Easy-Plex™ software records how many tips are left at the end of each run and this is recalled at the start of the next run. The Easy-Plex™ Processor will automatically move to the recorded first available tip location. If needed, the tip availability can be reset to three full racks by clicking Tools > Reset Tip Availability.

36.8.5 Prepare the Analyser Plate

Prior to loading into the High Plex 24, the 384 well Step 2 plate needs to be sealed with a real-time grade clear adhesive seal and spun in the plate spinner centrifuge.

- Refer to document MP9.31 (Ausdiagnostics High Plex 24 system package insert for instructions).

36.8.6 Load High-Plex Plate into MT-Analyser

1. The MT-Analyser can be opened in two ways:

I. Using the Analyser software in the Start run tab, select the Open thermal unit icon on the bottom right of the window. Select the Close thermal unit icon to close the Analyser.

or

II. Using the buttons underneath the Analyser screen, select the button on the left with the yellow light. This provides two options Exit (left button), or Open (middle button). Press the middle button once

2. Place the sealed STEP 2 PLATE in the Analyser.
3. To close the Analyser, press the left button to provide two options Exit (left button) and Close (middle button). Press the middle button once.

***IMPORTANT**: Ensure that the A1 well is placed to the top left corner and that all wells are correctly aligned within the analyser wells.*

The 384-well Analyser must be started within 30 minutes of the Processor finishing.

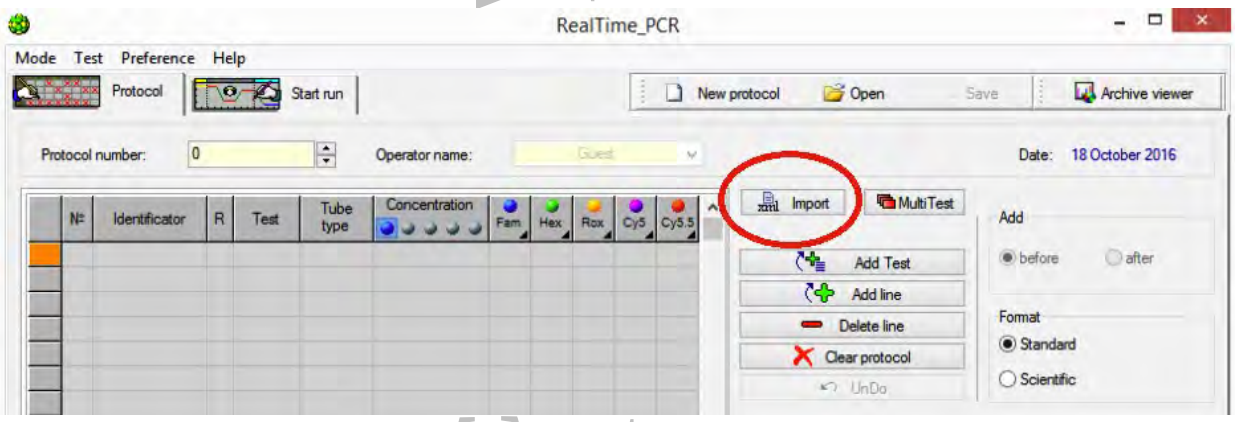
36.8.7 Start the MT-Analyser software (RealTime_PCR Program)

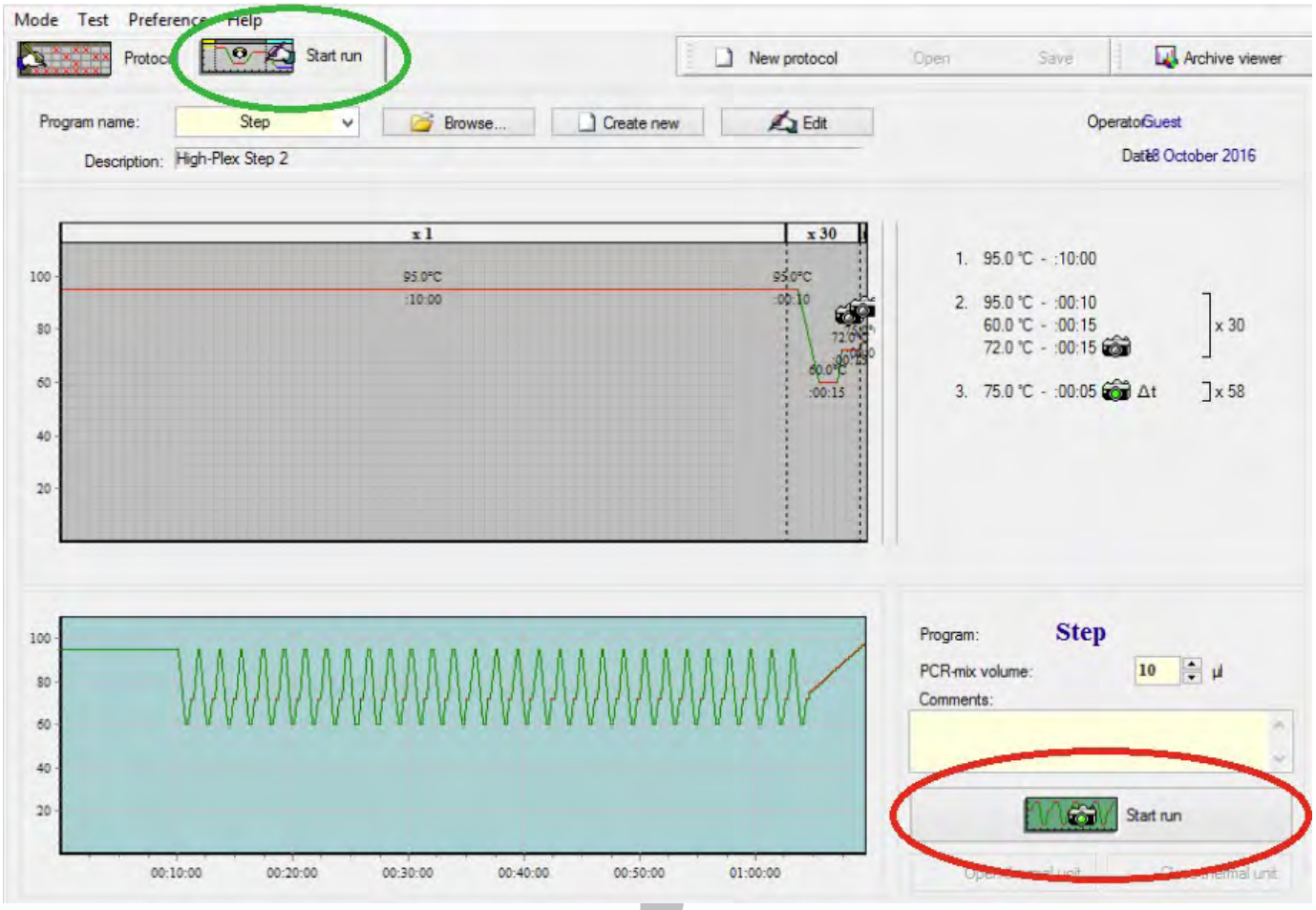
1. Ensure that the Analyser is switched on.
2. On the computer desktop, select the RealTime_PCR icon.
3. Select Operator as “ACT Molecular” and click *Device handling*.
4. A *List devices* window will appear. Choose which Analyser to use (the serial number can be found in the top left corner of the Analyser screen).
5. Select Connect.
6. The device will be connected to the computer. After automatic instrument testing, its status indication will be either:
7. Device is ON A green background, to indicate that the instrument is on and is ready for operation
8. Device is ON A yellow background, to indicate that the instrument has recently been switched on and will go through a warm-up (as displayed with a “WARMING” notice on the device screen if a run is initiated). After warm-up (max 10 min), the background colour will change from yellow to green.
9. Device is OFF A red background, to indicate that the instrument is either switched off at the moment of program startup, is not connected to the computer, has not been selected in the *List devices* window, or another instance of the software is open.
10. If the status indication is green or yellow, the **STEP 2 PLATE** may be loaded into the device

36.8.8 Run the MT-Analyser

If using the kit for the first time, see Section 8.1.4 Installing kit templates in MT-Analyser before proceeding any further.

1. Once the Analyser has been connected and is ready to use, select *Import*.
2. Select the correct Step 1 output file from the MT-Processor (.xml). Select *Open*.
3. The Analyser software will load the run setup from the imported file.
4. Select the *Apply* button.
5. In the Start run tab, select *Start run* to initialise the Step 2 PCR.





6. When the run is started (by clicking “Start Run”), a camera shutter sound will be heard accompanied by the following on-screen message. This indicates that the internal hardware diagnostics are being performed to ensure everything is working correctly.

7. The user will then be prompted to save the results files in the desired directory .xml) from the *C:\ Library\ Documents\ My Documents\ Ausdx HiPlex* directory or documents. Click Open & Save

***IMPORTANT**: If “Cancel” is selected on the Save Prompt, a warning message will ask whether the Starting Process should still be continued. If “Yes” is selected, the Step 2 PCR will proceed WITHOUT saving the run. However, a copy of the last performed run can always be found in C:\Program Files (x86)\DnaTechnology\RealTime_PCR\Current_Result.* The software tracks the cycling in real time.

8. After Step 2 has finished, an Attention! message box will prompt the user: “Would you like to open the file in MT Analysis software?”. Click YES to open the run file in the MT Analysis software. If NO is clicked, the standard DNA-Technology, post-run analysis loading screen will be displayed.

36.8.9 Instrument Clean Up Procedure

When the run has finished, **check volume of Step 1 tubes and make sure they are even to ensure no pipetting errors were encountered during the run** before the MT Assay Setup software adds bleach to the **STEP 1 TUBES**. After the **STEP 2 PLATE** has been removed, the **STEP 1 TUBES** can be bleached.

1. Select *Continue* for the Processor to add bleach to the **STEP 1 TUBES**.

2. After bleach has been added, dispose of the tubes and dilution plate in the waste bag.

3. Wipe any spillages on the surface of the MT-Processor deck (including the thermal cycler cover) with a non-corrosive nucleic acid denaturing reagent (e.g. DNA-OFFTM). DO NOT USE BLEACH TO CLEAN THE INSTRUMENT.

4. Remove the tip ejector chute and rinse any deposit away in running water to avoid bleach damage to the chute.

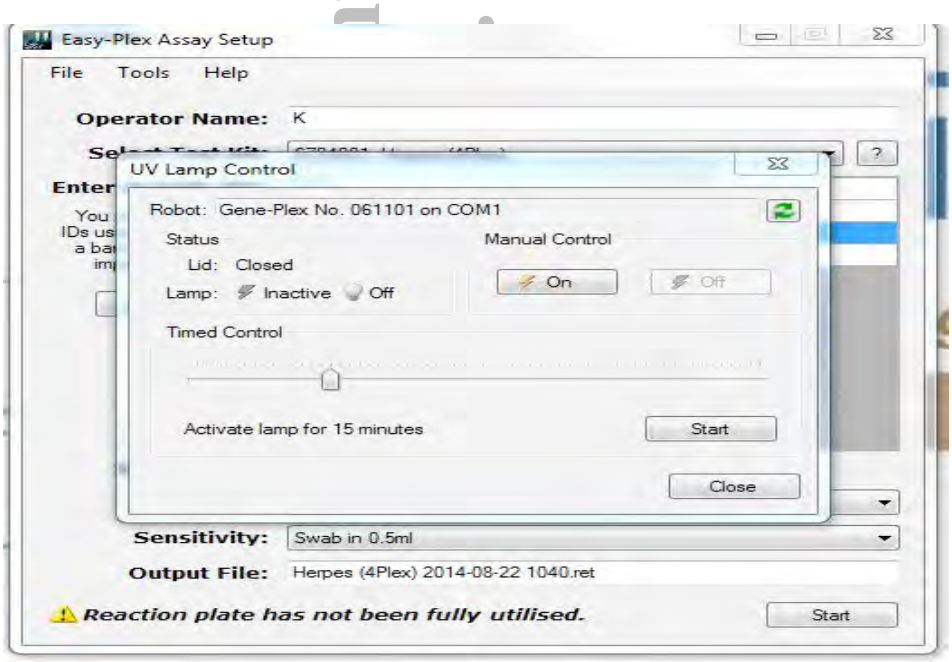
5. Seal and dispose of the waste bag according to safety regulations relevant for biohazard waste. One bag is provided for every two runs.

6. The Processor deck should be irradiated with the UV light between runs to inactivate any DNA material on the deck surface. Close the Processor lid before turning on the UV lamp. The UV lamp is turned on by selecting *Tools > UV Lamp Control.*

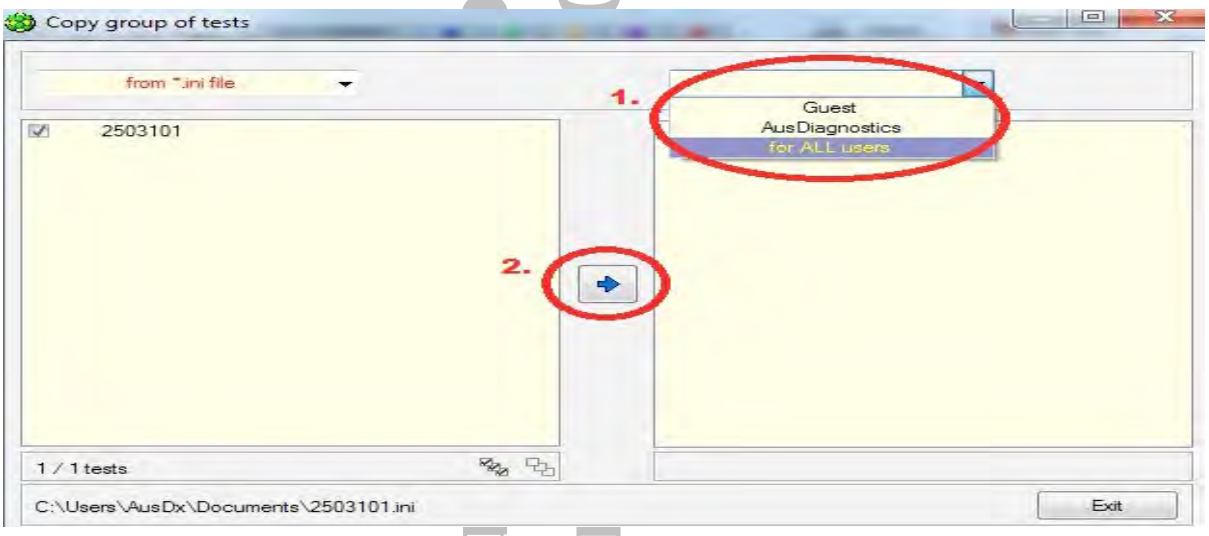
7. Select *Start* to turn on the UV lamp.

36.8.10 Installing Kit Templates

1. On the computer desktop, select the RealTime_PCR icon
2. Connect to the AusDiagnostics High-Plex Analyser:
3. Select *Tests* > *Copy group of tests*

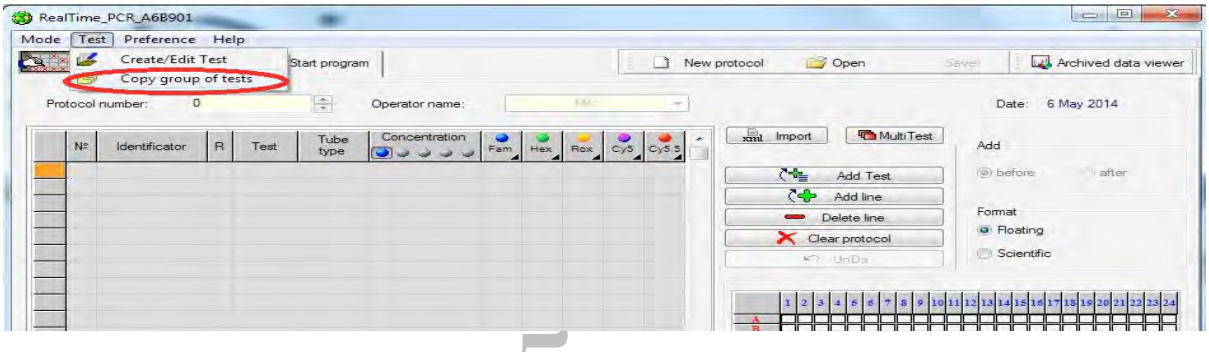


4. Click the from *.ini file from the drop-down menu

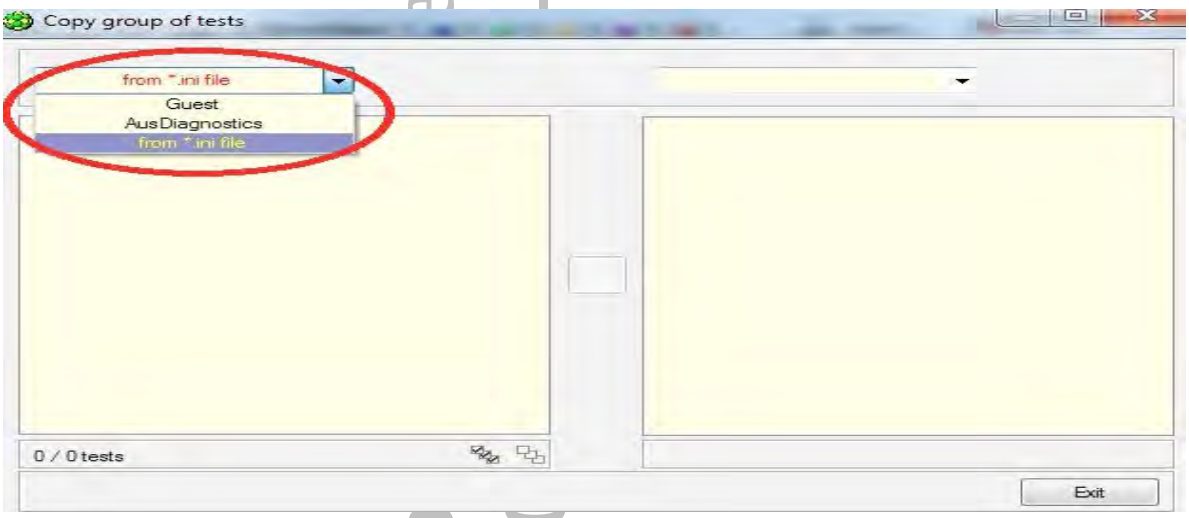


5. Go to the MT Assay Setup output directory. The location of the output directory can be retrieved by opening the MT Assay Setup software, and opening *Tools* > *Options*

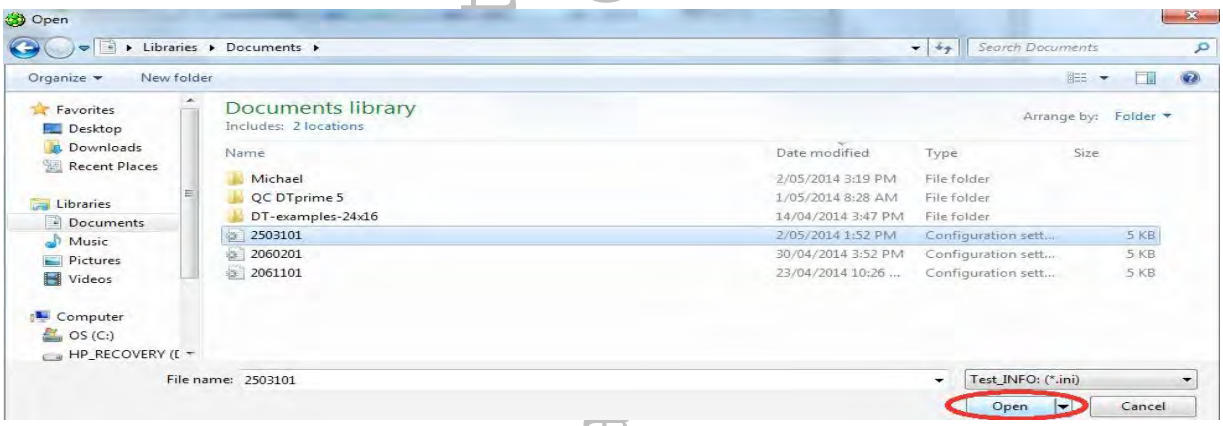
6. Select the *.ini file for the new kit template

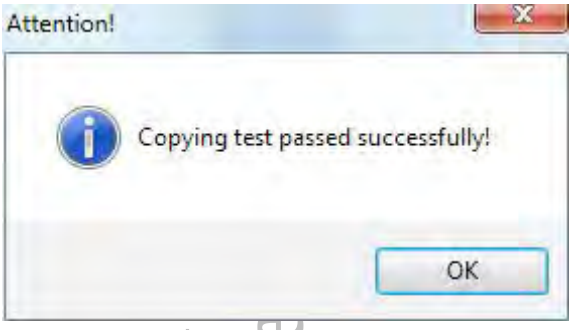


7. Tick the desired *.ini file, choose *for ALL users* (1) and then select the copy button (2).



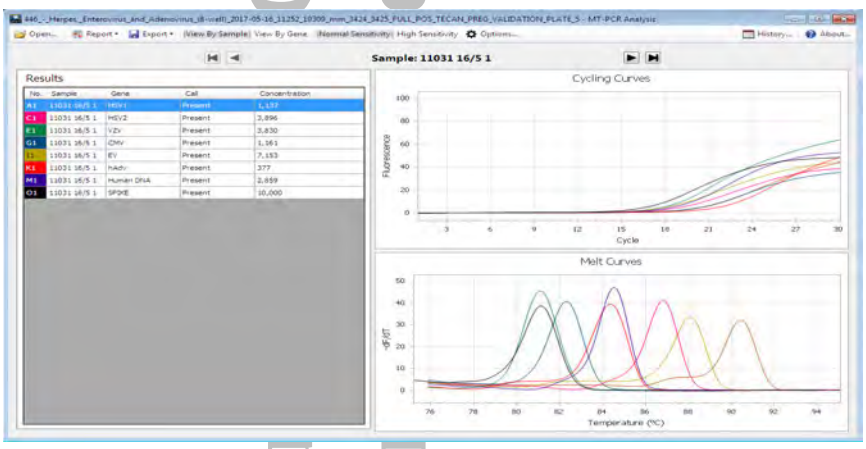
8. The new template is now installed and the *.xml files can be imported for that template.





36.8.11 Interpreting Results

1. Double click the MT-Plex™ Results software icon located on the desktop.
2. Select run to analyse.
3. Right click on concentration Tab & select Advanced view to obtain take off values.
4. Advanced View shows Corrected Melt, Take-Off values, Calculated Ct, Concentration, Rejection User and Rejection Comment.
5. Add 15 cycles Take Off Values to calculate the final crossing point value.
6. In the Results software, the cycling curves and the melt curves are displayed. Based on predefined parameters, the software will call the target as ‘Present’, ‘Check’ or blank (negative).
7. The results can be viewed by sample: The cycling curves (top) and the melt curves (bottom) for all the targets that have amplified are displayed for one sample. The expected melt temperature range for each target is displayed by a highlighted area.



8. The results can also be viewed by gene: With this setting, all of the samples that amplified per target are shown. The expected melt range is also highlighted per target.

Note the navigator buttons “<” and “>” which toggle the display between samples or genes.

36.8.12 Interpretation Glossary

36.8.12.1 Present

A target is considered present when the cycling curve displays amplification that falls within predetermined parameters. For some kits where dual infection is anticipated, the relative concentration of each positive gene is expressed as a percentage. The result is displayed in the Call column e.g. "Present (95%)". Percentages are rounded to the nearest 1% with <1% shown for smaller values.

36.8.12.2 Present (Low)

A concentration threshold can be implemented through the options function to distinguish low level from high level targets (see **Section 10.10 Options**). Any targets with a concentration lower than the set threshold, will be called “Present (Low)”. All report types will contain the text “Present (Low)” rather than“Present” to indicate operator involvement.

36.8.12.3 Blank

A target is considered undetected when there is no amplification within the predetermined parameters.

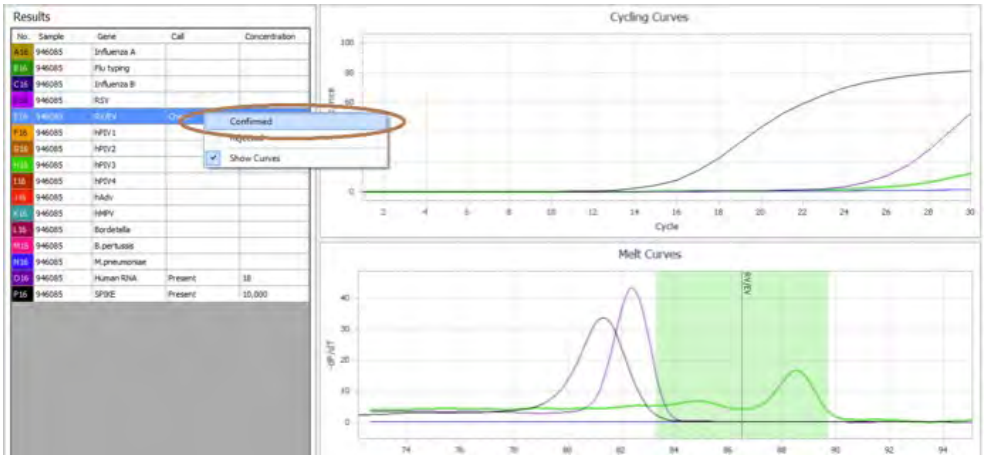
36.8.12.4 High Fluorescence

The high fluorescence condition is displayed when a well has a significantly higher fluorescence than the average fluorescence of all of the SPIKE reaction controls. This may indicate a high fluorescence particle (e.g. dust contamination) in the plate wells.

36.8.12.5 Check

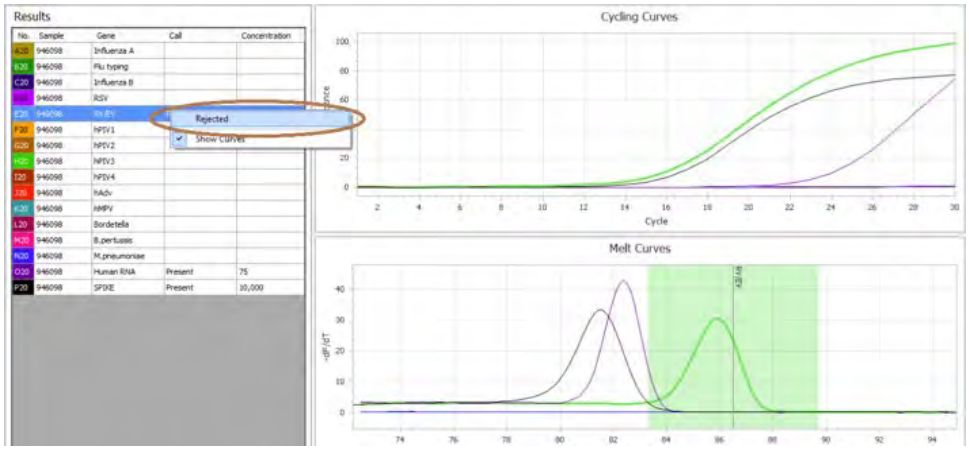
In some cases when the cycling curve acceleration is less than preset parameters, targets will be marked as “Check” rather than “Present”, and no concentration estimate is provided. The operator may right select on the “Check” call and change it to “Confirmed”. The normalised melt peak will remain visible in both the melt graph and in the sample report. All report types will contain the text “Confirmed” rather than “Present” to indicate operator involvement.

Note: During the determination of the clinical performance of IVDs, “Check” results were excluded from the analysis.

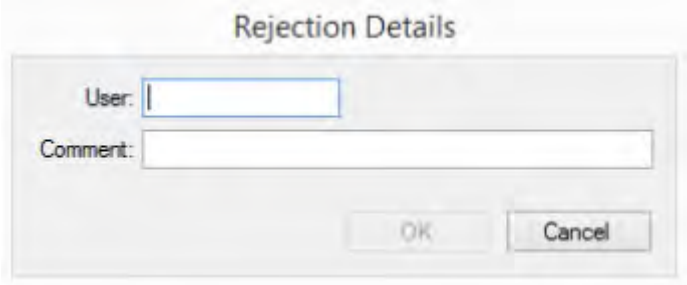


36.8.12.6 Rejection

A call may be rejected by right clicking the “Present” call and changing it to “Rejected”.



Upon rejecting a call, a message will prompt the operator to enter his or her name, and reason for rejecting the call. These details will subsequently be added to Analysis Report, and the call will change from “Present” to “Rejected” for that particular target.



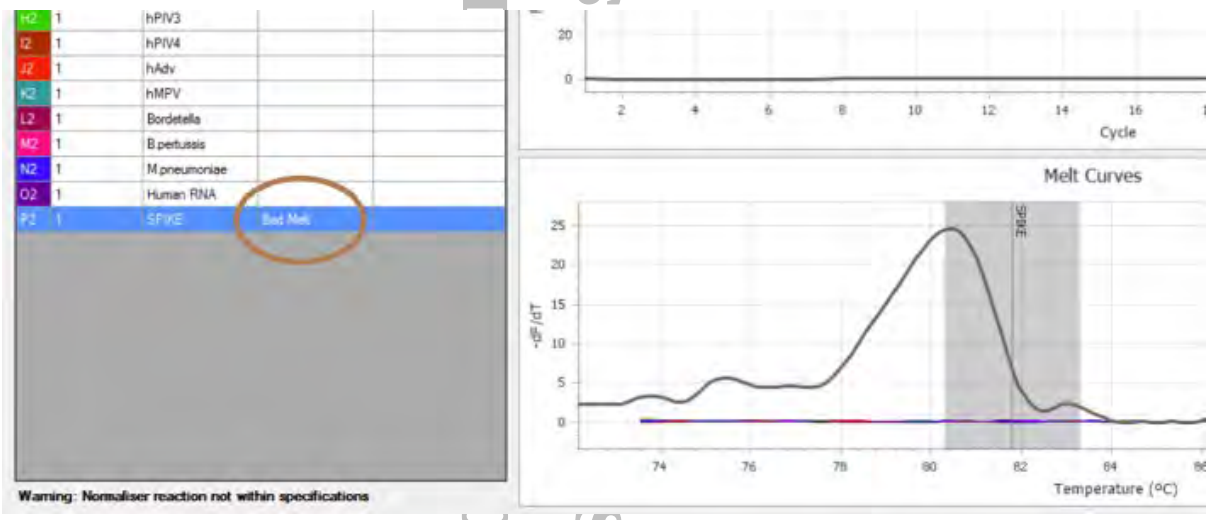
36.8.12.7 Inhibited

SPIKE, the internal control, is included in every assay. The detection of the SPIKE target controls for the integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cyclers) and the presence of inhibitors in the sample. The SPIKE cycling curves must take-off between 10-20 cycles with an S-shaped curve. If the SPIKE cycling curve has a take-off value greater than cycle

20, this indicates significant inhibition. A SPIKE take-off value greater than 5 cycles above the median is marked as *Inhibited*.

36.8.12.8 Poor Melting Results

If SPIKE's melt temperature is more than 0.5°C different from the mean, a "Bad Melt" is reported and the following warning message is displayed "Normaliser reaction not within specifications". This indicates an incorrect reaction mix and the sample should be retested.
If this recurs, contact AusDiagnostics.



36.8.12.9 Corrected Melt

Melt temperatures of all the different targets are adjusted according to the difference between the raw melting temperature and the known melting temperature of SPIKE (81.8°C). Corrected melts are used in the calling algorithm to allow for small changes in running conditions on individual systems.

36.8.12.10 Take Off Values

The take-off value is derived by stepping back from the point of maximum second differential, d2F/d2T, (i.e. maximum acceleration) to the point where the fluorescence emerges from background.

36.8.12.11 Concentration

Molecular target concentrations, expressed as arbitrary units, are calculated relative to the internal control SPIKE, which amplifies a known amount of target molecules. As the concentration of SPIKE is not measured these values are in arbitrary units.

36.8.12.12 Normal Sensitivity

In *Normal Sensitivity* the fluorescence melt temperature threshold for detection is set at 5% of the total fluorescence. Any targets with a fluorescence higher than this value will be displayed as normalised peaks.

36.8.12.13 High Sensitivity

In *High Sensitivity* the fluorescence melt temperature threshold for detection is lowered to 2% of the total fluorescence and lower acceleration rates are valid. Any targets with a fluorescence higher than this value that comply with expected melt and acceleration parameters will call present and be displayed as normalised peaks.

36.8.13 Data Recording on Worksheet.

1. Scroll through results of each patient by using the single left and right arrows in the table header.

2. Established sample validity by analysing the SPIKE and NONO results for each patient. Record all SPIKE positive results on your worksheet.

3. Record the crossing point value of all detected targets for each sample by adding 15 cycles to the take-off value. This value must be recorded for ongoing QC monitoring of the controls in this assay.

36.8.14 Quality Control

A synthetic positive control is included in **every** run and acts as a POSITIVE CONTROL for the assay. In addition to this, a positive extraction and negative extraction control are included in every run to monitor the extraction and set up process. QC results are entered in Kestral (refer to document MP1.08 for instructions). The synthetic positive QC sample is purchased from Ausdiagnostics.

For the synthetic positive control all targets including Human DNA (genomic DNA control) and SPIKE (artificial sequence for assay control) must be detected for the run to be valid. In addition, the positive and negative extraction control must pass the quality control criteria for the run to be valid.

In cases where one or more targets is NOT DETECTED, and the QC has failed, the assay is INVALID. No patient results contained within an INVALID run are acceptable for reporting.

36.8.15 Genomic DNA (Human DNA control) and Virus Target NOT DETECTED cases.

Whenever a NOT DETECTED viral target result is obtained on a patient sample which also gives no detectable genomic DNA (indicated by no signal in the Human DNA target), the sample should be re-extracted and repeated and if the Human DNA is still not detected the patient result is considered VALID but is reported with a NEGNONO comment below:

*"This report should be interpreted with **CAUTION**. The human DNA detection control was NEGATIVE in this test suggesting an insufficient specimen collection. Therefore, this result cannot exclude the presence of any viral pathogens tested in this assay for this patient with high certainty. **Recollection is recommended"**.*

36.8.16 Interpretation Criteria

The selection criteria for interpretation of results are:

DETECTED
Targets with a distinct amplification curve and take-off value within the range 2 to 20 (total 17 – 35) are reported as detected.

INDETERMINATE
Targets with a distinct amplification curve and take-off value within the range 21 to 23 (total 36 – 38) must be interpreted as indeterminate with the appropriate comment.

NOT DETECTED
Targets with no distinct amplification curve with no take-off value are reported as not detected.

36.9 Internal Quality Control

A commercially available positive control is included which contains all targets.

An extraction QC sample is tested each run with data recorded in LIMS. The data is reviewed every six months.

36.10 EQAP

This assay is checked for performance by participating in quality assurance programs primarily from the Royal College of Pathologists Quality Assurance Programs (RCPA QAP).

36.11 Method Limitations

The manufacturer reports the following limitations.

- This product is only for use by suitably trained personnel in qualified laboratories.

- The performance of the SARS-CoV-2, Influenza and RSV 8-well panel has only been established on the AusDiagnostics High-Plex 24 systems.

- The assays in this product do not provide a quantitative value for the pathogen(s) in the sample.

- The performance of the test has been evaluated for use with human specimen material only.

- The performance of this test has only been validated with nucleic acid extracts from the following specimen types: nasal swab, throat swab, nasopharyngeal swab, nasopharyngeal aspirate (NPA), tracheal aspiration, bronchial washing, and bronchoalveolar lavage (BAL). It has not been validated for use with other sample types.

- The performance of this test has not been established for patients without symptoms of respiratory illness.

- The performance of this test has not been established for immunocompromised individuals.

- The SARS-CoV-2, Influenza and RSV 8-well panel is designed to measure specific nucleic acid sequences. Therefore, a negative result does not exclude the possibility that an unusual sequence variant is present. The results obtained with this product should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. A negative result cannot be relied upon for a definitive diagnosis. Failure or delay in treatment of an infected patient may lead to death, with immunocompromised patients being at highest risk.

- The detection of nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (using manufacturer of specimen collection devices instructions). Failure to follow proper procedures can lead to incorrect results.

- Discrimination between RSV A and RSV B strains is for information purposes only.

36.12 Clinically Significant Interferences

All SARS-COV-2 and other virus positive cases are reviewed by a pathologist.

36.13 Potential Sources of Variability

36.13.1 Sample type and storage

Patient sample type, collection and storage conditions may affect assay performance.

36.13.2 Interfering substances

Interfering substances have not been tested in the laboratory for this test.

The manufacturer states a range of exogenous and endogenous substances including those expected to be found in the sample types for this panel were tested for potential PCR interference. Minimal or

no interference was seen due to the presence of any one of the substances tested. The presence of the internal control, SPIKE, in all AusDiagnostics panels controls for possible PCR interference in each sample.

36.14 Clinical Significance

This test was installed in response to the 2020 SARS-COV2 outbreak.

36.15 Action Results

For cases with low viral loads, a separate confirmatory assay or referral may be required.

36.16 Critical Results

Medical Officers are routinely notified of positive results. Other notifications occur on a case by case basis.

36.17 Kestral Alt-9 Information

Alt-9 information entered under code “CoronaPCR.”

36.18 Safety Precautions

All samples must be handled carefully as though infectious.
Safe handling practices including Personal Protective Equipment (PPE) must be utilised when handling specimens, reagents and operating instrumentation.

36.19 EQAP

The laboratory is enrolled in a quality assurance program provided by Royal College of Pathologists QAP. The name of the program is PHLN Module: SARS-CoV-2 and other coronaviruses.

36.20 Records

Report Examples

NATA Accreditation Numbers
TCH #2508 Calvary #15024

ACT Pathology

PO BOX 11, WODEN ACT 2606
Ph (02) 5124 2930 FAX (02) 5124 2962

Copies to:

Requesting Dr: CLIN CHEM QC OFFICER

Report to:
TCH Act Pathology
Po Box 11
Woden 2606

Referred By : Clin Chem QC Officer

ACTPMI: not avail.

SURNAME: KESTRAL
GIVEN: BABY
DOB: 26/10/2019 **SEX:** Male
ADDRESS: Gilmore Crescent
GARRAN 2600

Collect Date: 29/04/2020 Time: *UNK* Specimen No: 20P141271
Receive Date: 29/04/2020 Time:00:00
Report Date: !UNAUTHORISED REPORT!

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SAR-CoV-2)	**Not Detected**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**Not Detected**
Respiratory syncytial virus (RSV)	**Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

MOLECULAR PATHOLOGY

Specialists - Drs Karina Kennedy, Chong Ong, Peter Collignon, Heather Wilson, Anindita Das & Gary Lum. Ph: (02) 5124 3485
Clinical Notes: Testing !!!!!UNAUTHORISED REPORT!!!!!
EUC,CORPCR
Director: Professor Jane Dahlstrom (APP) Provider No. 227762B
!!!!!UNAUTHORISED REPORT!!!!! Printed: 08/05/2020 16:41

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
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Referred By : Clin Chem QC Officer

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SAR-CoV-2)	**DETECTED**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**Not Detected**
Respiratory syncytial virus (RSV)	**Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, has been DETECTED.

Please refer to Public Health and Infection Prevention and Control advice regarding patient management and management of contacts.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

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Printed: 08/05/2020 16:42

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Testing Performed at: TCH Laboratory
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Do

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TCH #2508 Calvary #15024

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Receive Date: 29/04/2020 Time: 00:00
Report Date: !UNAUTHORISED REPORT!

Referred By : Clin Chem QC Officer

Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SAR-CoV-2)	**Indeterminate**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**Not Detected**
Respiratory syncytial virus (RSV)	**Not Detected**

An INDETERMINATE result was obtained.

This may be due to low numbers of the novel coronavirus in the specimen or non-specific amplification of related viruses.

Interpretation of the results should be based on epidemiological risk and clinical features. Consideration should be made to repeat specimen collection. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

For further advice regarding this test result please contact the on-call Clinical Microbiologist via Canberra Hospital switch on 5124 0000.

This test has not been fully validated to the current NPAAC guidelines and results should be interpreted accordingly.

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Molecular Studies

Specimen Type: **Flocked Swab in VTM**
Site: **Nasal**

TEST: Coronavirus Nucleic Acid Testing

METHOD: Real-time PCR

Coronavirus (SAR-CoV-2)	**Not Detected**
Influenza A	**Not Detected**
Influenza A Typing	**Not Detected**
Influenza B	**DETECTED**
Respiratory syncytial virus (RSV)	**Not Detected**

Coronavirus (SARS-CoV-2), the causative agent of COVID-19, was NOT detected.

If there is a strong clinical suspicion of infection, particularly if the specimens were taken early in the illness, then repeat testing may be indicated. Lower respiratory tract specimens appear to be more sensitive than upper respiratory tract specimens.

If this patient is in self-isolation or quarantine for a defined period of time due to overseas travel or due to contact with a confirmed COVID-19 case, then a negative result on this test does not exempt this patient from completing the required period of self-isolation or quarantine. For further details, please contact your local Public Health Department.

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Printed: 08/05/2020 16:42

Accredited for compliance with NPAAC Standards and ISO 15189.
Testing Performed at: TCH Laboratory
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Do

36.21 Associated Documentation

1. MP1.08 QC and QA Specimens and Procedures.
2. MP10.03 Automated Liquid Handling- QIAgility Robot.
3. MP05.11 Nucleic Acid Extraction on Roche MagNA Pure Systems
4. MP9.31 Ausdiagnostics High Plex 24 System
5. MP2.127 Competency Appraisal – Viral B Bench

36.22 References

Parliament, E., Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (Text with EEA relevance), in 1272. 2008.

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Lau, A., et al., Colony multiplex-tandem PCR for rapid, accurate identification of fungal cultures. J Clin Microbiol, 2008. 46(12): p. 4058-60.