MICROBIOLOGICAL QUALITY OF READY-TO-EAT FOODS

ACT HEALTH PROTECTION SERVICE



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BACKGROUND/OBJECTIVE

Ready-to-Eat (RTE) food is food that is ordinarily consumed in the same condition in which it is sold or distributed and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumers.

Sandwiches, rolls, stir-fries, baked goods as well as various other RTE foods are widely available in the Australian Capital Territory (ACT) with approximately 250 different licensed outlets. Due to the diverse nature and popularity of these foods it was considered prudent to perform ongoing surveys on these products. The survey of RTE products was undertaken for three main reasons:

- To determine the bacteriological status of ready-to-eat food products available on the ACT market.
- 2. To determine the compliance of these products to Food Standards Australia New Zealand (FSANZ) Guidelines for the Microbiological Examination of Ready-to-Eat (RTE) Foods 2001 (FSANZ RTE Guidelines).
- 3. To complement and focus inspections of high-risk food producing establishments.

STANDARDS

The FSANZ RTE Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guidelines. Table 1 not only reflects both the high level of microbiological quality that is achievable for RTE foods in Australia and New Zealand but also indicates the level of contamination that is considered to be a significant risk to public health.

Table 1¹

Table I				
Test	Microbio		(Colony Forming (CFU/g))	Units per
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Standard Plate Count (SPC	()			
Level 1*	<10 ⁴	<10 ⁵	≥10 ⁵	
Level 2*	<10 ⁶	<10 ⁷	≥10 ⁷	
Level 3*	N/A	N/A	N/A	
Indicators				
Escherichia coli (E. coli)	<3	3-100	>100	**
Pathogens				
Coagulase positive	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
staphylococci (Staph)				SET +ve
Bacillus cereus (B. cerues)	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
Salmonella spp.	not detected			detected
	in 25g			
Listeria monocytogenes	not detected	detected but		≥10 ^{2 ##}
(L. monocytogenes)	in 25g	<10 ^{2 #}		

NOTE

^{*}see below "Standard Plate Counts" for definition of level.

^{**} Pathogenic strains of E. coli should be absent.

Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g. ## The detection of *L. mono* in ready-to-eat-foods prepared specifically for "at risk" population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous. SET +ve: Staphylococcus enterotoxin positive.

N/A – SPC testing not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls).

Level 1 – applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process/preparation of the final food product and, as such, microbial counts should be low i.e. fried chicken.

Level 2 – applies to ready-to-eat foods which contain some components which have been cooked and then further handled (stored, sliced or mixed) prior to preparation of the final food or where no cooking process has been used i.e. custard slice.

Level 3 – SPC not applicable. This applies to foods such as fresh fruits and vegetables (including salad vegetables), fermented foods and foods incorporating these (such as sandwiches and filled rolls). It would be expected that these foods would have an inherent high SPC because of the normal microbial flora present

An examination of the microbiological quality of a food should not be based on SPC alone. The significance of high (unsatisfactory) SPC cannot truly be made without identifying the predominant microorganisms or other microbiological testing.

SURVEY

This survey was conducted between July 2009 and June 2010. A total of eighty six samples were collected from twenty two different ACT retail outlets. The samples were randomly collected by the Health Protection Service (HPS) Public Health Officer (PHO) and processed by the Microbiology Unit of the HPS. The survey collected multiple samples from single outlets and in general outlets were only tested once.

The samples were collected in such a manner as to cover a wide range of the available ready to eat food ranging from dips to grilled chicken. All of the samples were tested for the hygiene indicators SPC and *E. coli*, and the food pathogens coagulase positive *Staphylococci*, Salmonella and *L. monocytogenes*. Foods containing pasta or rice were also tested for *B. cereus*.

Marginal results may be re-sampled; this is dependent on resources as these foods are still considered compliant. Where the HPS identifies non compliance issues in food businesses, corrective actions are addressed through a graduated and proportionate response. Unsatisfactory results are re-sampled; if the food item is not available other food items may tested. Unsatisfactory SPC results are not re-sampled unless pathogens are also isolated.

MICROBIOLOGICAL METHOD OF ANALYSIS

- Salmonella AS 1766.2.5 1991 (modified).
- SPC AS 5013.5 2004.
- B. cereus AS 5013.2 2007.
- Coagulase positive *staphylococci* AS 5013.12.2 2004.
- E. coli ISO 16649.2 2001.
- *L. monocytogenes AS* 1766.2.16.1 1998 (modified).

The sample preparation for SPC, *E. coli*, coagulase positive *staphylococci* and *B. cereus* consisted of:

- 25g of sample being homogenised with 225mL of 0.1% peptone diluent
- subsequent serial dilutions were prepared for use in enumeration.

B. cereus enumeration: Spread plates (using a 100µl of each dilution) on a solid selective medium containing egg yolk and mannitol (MYP agar). Typical large, pink colonies, with or without lecithinase action were counted and a proportion of the colonies confirmed by a haemolysis test and spore staining. **B. cereus** cells are rods 4-5 μ m long and 1-1.5 μ m wide and stain red. The cells contain black-stained lipid globules. The spores stain green, are ellipsoidal in shape, central to sub central in position, and do not swell the sporangium.

Coagulase positive *Staphylococci* **enumeration:** Pour plates (using 1.0 ml of each dilution) of Baird Parker medium with rabbit plasma fibrinogen added were prepared in duplicate and incubated at 37°C/48h. Typical black colonies, surrounded by a halo of precipitation, were counted.

E. coli enumeration: Pour plates using 1.0 ml in each plate of TBX media were prepared and incubated at 37 degrees Celsius for 4 hours and then 44 degrees Celsius for 18-24 hours. Typical blue/green colonies were counted.

Salmonella detection: 25g of sample was weighed out aseptically and homogenised with 225mL buffered peptone water (non-selective enrichment) and incubated at 37°C/16-20h. Aliquots were then transferred into Brain Heart Infusion broth (BHI) and incubated for 3h. DNA was extracted from 200uL of enriched BHI. This was screened for the presence of salmonella using a BAX cyber green Polymerase Chain Reaction (PCR) and a BAX Q7. No confirmation testing was performed as there were no samples that screened positive.

SPC: Pour plates (using a 1.0ml of each dilution or 0.1ml at the -6 dilution) of plate count agar where incubated at 30 °C/72h. Plates from the dilution on which there are greater 15 and less than 300 colonies visible were counted. Counts outside this range were considered estimate counts only.

L. monocytogenes detection: 25g of sample was weighed out aseptically and homogenised with 225mL half fraser broth (selective enrichment) and incubated at 30°C/24h. Aliquots were then transferred into a single tube of Fraser broth incubated for 37°C/24h and MOPS BLEB broth incubated for 37°C/24h. DNA was extracted from 200uL of enriched MOPS BLEB broth. This was screened for the presence of *L. monocytogenes* using a BAX cyber green PCR and a BAX Q7. No confirmation testing was performed as there were no samples that screened positive.

RESULTS

Test	Coagulase positive staphylococci (n=86)	L. monocytogenes (n=86)	Salmonella (n=86)	E. coli (n=86)	SPC (n=86)	B. cereus (n=42)
Number of marginal samples	5	Nil	N/A	3	Nil	Nil
Number of unsatisfactor y samples	Nil	Nil	Nil	1	4	Nil

Detailed results are tabled in Appendix B.

DISCUSSION

SPC

All samples (86) were tested for SPC. The results for all the samples ranged between <50 and 6.6 x 10⁸ colony forming units per gram (cfu/g). Twenty two samples were assessed as being in the Level 1 category and were found satisfactory with a range of counts between <50 to 5000 cfu/g.

Seventeen samples were assessed as being in the level 2 category. The results for these products ranged from <50 to 9.6 x 10⁷ cfu/g. No samples were in the marginal category. Four samples (4.6% of the total SPC test) were in the unsatisfactory category: Quiche Pumpkin, Chicken Mushroom Pasta, Alfredo Tortellini and Lasagne and Creamy Potato. High SPC for cooked products suggests that the handling or storage of these foods may have been comprised. No re-samples were taken of these foods as no pathogens or *E. coli* were detected at the time of testing.

A total forty seven samples were assessed as applying to the Level 3 SPC criterion. The SPC test is not applicable to these products. The results for these products ranged from 50 to 6.6×10^8 cfu/g.

E. coli

All samples (86) were tested for *E. coli*. The presence of *E. coli* in RTE foods is undesirable because it indicates that the food has possibly been prepared under poor hygienic conditions. Ideally *E. coli* should not be detected and as such a level of <3 cfu/g has been set for satisfactory samples. Eighty two (95.3%) samples tested in this survey had <3 cfu/g of *E. coli* and met the satisfactory criterion. There were three (3.5%) samples in the marginal category and one (1.2%) sample, a chicken sushi roll, in the unsatisfactory category. Follow-up action and re-samples were taken for the unsatisfactory result. The re-samples reported marginal results. A further five resamples were taken after the advised corrective action. All five re-samples reported satisfactory results. The detection of *E. coli* in foods is not a direct indication that the food is unsafe rather it is an indication of potential problems involving the preparing and handling of foods.

Coagulase positive Staphylococci

All samples (86) samples were tested for coagulase positive *Staphylococci*. Eighty one (94.2%) samples tested in this survey had met the satisfactory criterion i.e. <100 cfu/g, with five samples (5.8%) in the marginal category and none were unsatisfactory. Chicken sushi and salmon sushi marginal samples where retested as part of the *E. coli* re-sampling. The re-samples reported satisfactory counts of <50 cfu/g.

Salmonella

Salmonella was not detected in any of the eighty six samples tested. RTE foods should be free of Salmonella as consumption of food containing this pathogen may result in food borne illness.

Listeria monocytogenes

Foods in which all components have been cooked in the final food preparation, or have received some other listericidal treatment, should be free of *L. monocytogenes*. The detection of *L. monocytogenes* in foods indicates the food was inadequately prepared or the food was contaminated post preparation. The detection of higher levels (>10² cfu/g) of *L. monocytogenes* in RTE foods indicates a failure of food handling controls and is also considered a public health risk.

All RTE foods are tested for the presence of *L. monocytogenes* in 25g. If *L. monocytogenes* is detected PHO will inspect the premises and collect a resample of the food item if available. This re-sample will be tested semi-quantitatively to measure the level of *L. monocytogenes* in the food.

Eighty six samples were analysed for *L. monocytogenes*. All eighty six (100%) of the samples were satisfactory as *L. monocytogenes* was not detected.

B. cereus (Tested for in RTE foods containing rice or pasta only)

Forty two samples containing rice or pasta were tested for *B. cereus*. All forty two samples (100%) tested were satisfactory reporting counts of less than 100cfu/g.

CONCLUSION

The microbiological quality of the RTE foods surveyed in the ACT is good. Overall the results are comparable to those found in the previous five years (Appendix A). Raw results of the analysis are attached at Appendix B. SPC results have seen an improvement in satisfactory results for level one samples but a decrease in level two samples. This could indicate poor temperature control or age of products sold.

The overall results for pathogens have generally improved on previous years and have demonstrated the highest level of compliance to date. The percentage of satisfactory samples for *L. monocytogenes, E. coli* and *B. cereus* are the highest ever. The percentage of satisfactory samples for *Salmonella* has been very consistent, with none isolated in the last five years. Coagulase positive *Staphylococci* results have shown an increase in marginal results from the previous year. In conclusion, the results of this survey show a very high level of compliance with the FSANZ RTE Guidelines.

BIBLIOGRAPHY

Guidelines for the microbiological examination of ready-to-eat foods, FSANZ Dec 2001.

Microbiological Quality of Ready-To-Eat Foods 2005-2006, ACT Health Protection Service.

Microbiological Quality of Ready-To-Eat Foods 2006-2007, ACT Health Protection Service.

Microbiological Quality of Ready-To-Eat Foods 2007-2008, ACT Health Protection Service.

Microbiological Quality of Ready-To-Eat Foods 2008-2009, ACT Health Protection Service.

Foodborne Microorganisms of Public Health Significance, AIFST Inc. Food Microbiology Group.

APPENDIX A

COMPARISON TO PREVIOUS SURVEYS: 2005–2006, 2006–2007, 2007–2008, 2008–2009 and 2009–2010

One can be seen from Tables 1 and 2 below that the quality of RTE foods varies depending on the test. The percentage of satisfactory samples in both the SPC Level 1 and 2 categories have improved since the 2006-7 year. SPC results for 2009-2010 has seen improvement in satisfactory results for level one samples but a decrease in level two samples. Whereas results for pathogens tested has improved on previous years.

Table 1

Comparison of Standard Plate Counts (rounded)

Compan	Companison of Standard Flate Counts (rounded)														
%		S	Satisfactory Marginal							Unsatisfactory					
Year	05-06	06-07	07-08	08-09	09-10	05-06	06-07	07-08	08-09	09-10	05-06	06-07	07-08	08-09	09-10
Level 1	86.0	95.0	98.6	90.0	100	3.0	5.0	1.4	8.0	0.0	11.0	0.0	0.0	2.0	0.0
Level 2	87.0	88.0	91.1	90.0	76.5	7.0	12.0	5.1	6.0	0.0	7.0	0.0	3.8	4.0	23.5
Laval 3															

Table 2

%	2005-2006			2006-2007			2007-2008			2008-2009			2009-2010							
	Sat	Marg	Unsat	Pot. Haz	Sat	Marg	Unsat	Pot. Haz	Sat	Marg	Unsat	Pot. Haz	Sat	Marg	Unsat	Pot. Haz	Sat	Marg	Unsat	Pot. Haz
E. coli	92.0	6.0	2.0		86	11.0	3.0		93.1	6.5	0.4		94.4	5.0	0.6		95.3	3.5	1.2	
Coagulase +ve Staphylococcus	98.5	1.5	0.0	0.0	84	10.1	4.8	3.0	99.1	0.9	0.0	0.0	100	0.0	0.0	0.0	94.2	5.8	0.0	0.0
Salmonella spp.	100			0.0	100			0.0	100			0.0	100			0.0	100			0.0
L. monocytogenes	98.5	1.5		0.0	97.8	2.2		0.0	99.6	0.4		0.0	100	0.0		0.0	100	0.0		0.0
Bacillus cereus	92.5	2.5	0.0	2.5	88.0	12.0	0.0	0.0	93.1	6.9	0.0	0.0	92.0	8.0	0.0	0.0	100	0.0	0.0	0.0

Comparison between the Microbiological Quality indicators

Sat – Satisfactory, Unsat – Unsatisfactory, Marg – Marginal, Pot. Haz – Potentially Hazardous.

Not applicable

Appendix B

Appendix B												
Sample	level	SPC	E. coli	Staph	Salmonella	L. monocytogenes	B. cereus	Assessment				
Seafood salad	3	4500000	<3	<50	Absent	Absent	NA	S				
Potato, Egg and Bacon salad	2	6000	<3	<50	Absent	Absent	NA	S				
Hummus dip	2	200000	<3	<50	Absent	Absent	NA	S				
Fruit salad	3	180000	<3	<50	Absent	Absent	NA	S				
BBQ chicken	1	100*	<3	<50	Absent	Absent	NA	S				
Lemon Chicken	1	50*	<3	<50	Absent	Absent	NA	S				
Pork Spring rolls	1	<50	<3	<50	Absent	Absent	NA	S				
Fried Dim Sims	1	400*	<3	<50	Absent	Absent	NA	S				
Mongolian Beef	1	50*	<3	<50	Absent	Absent	NA	S				
Cashew Chicken	1	50*	<3	<50	Absent	Absent	NA	S				
Half Chook	1	<50	<3	<50	Absent	Absent	NA	S				
Potato salad	2	450*	<3	<50	Absent	Absent	NA	S				
Tabouleh	3	30000*	<3	<50	Absent	Absent	NA	S				
Couscous/roasted	_		_					_				
Pumpkin	3	14000000	<3	<50	Absent	Absent	<50	S				
Coleslaw Chicken green	3	130000	<3	<50	Absent	Absent	NA	S				
curry with rice Chicken Korma	1	1400	<3	<50	Absent	Absent	<50	S				
with rice Fried Chicken	1	<5000	<3	<50	Absent	Absent	<50	S				
wings	1	5000*	<3	250	Absent	Absent	NA	М				
Beef Curry rolls	1	<5000	<3	100	Absent	Absent	NA	M				
Beef Rice Paper	_		_									
rolls	3	5000000*	<3	<50	Absent	Absent	<50	S				
Thai Beef salad BBQ Chicken	3	670000	<3	<50	Absent	Absent	NA	S				
salad	3	920000	<3	<50	Absent	Absent	NA	S				
Caesar salad	3	1100000	<3	<50	Absent	Absent	NA	S				
Quiche Pumpkin	2	19000000	<3	<50	Absent	Absent	NA	U				
Quiche Bacon	2	85000*	<3	<50	Absent	Absent	NA	S				
Lamb Rogan Josh with rice	1	<50	<3	<50	Absent	Absent	<50	S				
Butter Chicken with rice	1	150*	<3	<50	Absent	Absent	<50	S				
Chicken Tika Marsala	1	50*	<3	<50	Absent	Absent	NA	S				
Curry Potato and Peas	1	<50	<3	<50	Absent	Absent	NA	S				
Samousa	1	5000*	<3	<50	Absent	Absent	NA	S				
Lettuce mix	3	400000	<3	<50	Absent	Absent	NA	S				
Fruit mix	3	23000	<3	<50	Absent	Absent	NA	S				
Custard tart	2	250*	<3	<50	Absent	Absent	NA	S				
Tuna and Corn Pasta salad	3	9600	<3	<50	Absent	Absent	<50	S				
Tabouli	3	4500*	<3	<50	Absent	Absent	<50	S				
Chicken risotto	3	110000000	<3	<50	Absent	Absent	<50	S				
Sundried Tomato and Vegetable	3	88000000	<3	<50	Absent	Absent	<50	S				
Chicken Mushroom Pasta	2	96000000	<3	<50	Absent	Absent	<50	U				
Alfredo Tortellini	2	10000000	<3	<50	Absent	Absent	<50	U				

Sample	level	SPC	E. coli	Staph	Salmonella	L. monocytogenes	B. cereus	Assessment
Lasagne and								
Creamy Potato	2	57000000	<3	<50	Absent	Absent	<50	U
Flame Grilled Chicken	1	<50	<3	<50	Absent	Absent	NA	S
Spicy Chicken	1	<50	<3	<50	Absent	Absent	NA	S
Greek salad	3	24000000	<3	<50	Absent	Absent	NA	S
Molotof	1	<50	<3	<50	Absent	Absent	NA	S
Fruit salad	3	120000000	<3	<50	Absent	Absent	NA	S
Sushi Rice rolls with Chicken	3	24000000	<3	<50	Absent	Absent	<50	S
Honey Chicken with rice	1	150*	<3	<50	Absent	Absent	<50	S
Singapore						Alexand		
noodles Chicken with	1	1500*	<3	<50	Absent	Absent	<50	S
Cashew nut and rice	1	50*	<3	<50	Absent	Absent	<50	S
Satay Chicken and Rice	1	<5000	<3	<50	Absent	Absent	<50	S
Fruit salad	3	1500000	<3	<50	Absent	Absent	NA	S
Chicken roll	3	15000000	<3	<50	Absent	Absent	NA	S
Chicken Roll Cheese roll	3	15000000	<3	<50	Absent	Absent	NA	S
Turkey Wrap	3	9000000*	<3	<50	Absent	Absent	NA	S
Chicken Schnitzel toll	3	19000000	<3	<50	Absent	Absent	NA	S
Tuna and Pasta salad	3	55000*	<3	<50	Absent	Absent	<50	S
Rice and Lentil salad	3	1700000	<3	<50	Absent	Absent	NA	S
Caesar salad	3	100000*	<3	<50	Absent	Absent	NA	S
Potato salad	3	400000*	<3	<50	Absent	Absent	NA	S
Italian salad	3	260000	<3	<50	Absent	Absent	NA	S
Ham and Mayo twirl	2	50*	<3	<50	Absent	Absent	NA	S
Magherita Pizza slice	2	600*	<3	<50	Absent	Absent	NA	S
Chicken Sushi roll	3	23000000	640	600	Absent	Absent	<50	U
Beef Sushi roll	3	20000000	27	850	Absent	Absent	<50	M
Salmon Sushi roll	3	12000000*	7	50	Absent	Absent	<50	M
Spanish Onion and Bococcini pie	2	20000*	<3	<50	Absent	Absent	NA NA	S
Cabbage roll	3	26000000	<3	<50	Absent	Absent	NA	S
Hummus dip	2	290000	<3	<50	Absent	Absent	<50	S
Mango		230000	\ 0	\ 00	Absent	Absont	\ 30	Ü
Passionfruit yoghurt	3	95000000	<3	<50	Absent	Absent	<50	S
Tzatziki dip	3	100*	<3	<50	Absent	Absent	<50	S
German Potato salad	3	2200	<3	<50	Absent	Absent	50	S
Fruit yoghourt	3	48000000	<3	<50	Absent	Absent	<50	S
Baby Octopus	3	660000000	<3	<50	Absent	Absent	<50	S
Beetroot dip	3	14000	<3	<50	Absent	Absent	<50	S

Sample	level	SPC	E. coli	Staph	Salmonella	L. monocytogenes	B. cereus	Assessment
Thick & Fruity						, ,		
Passionfruit Yoghurt	3	5000*	<3	<50	Absent	Absent	<50	S
Dairy Yoghurt					7 1200111	, 1000. II		
Berry Twist	3	1600	<3	<50	Absent	Absent	<50	S
Tzatziki Dip	3	<5000	<3	<50	Absent	Absent	<50	S
Tzatziki Dip	3	300*	<3	<50	Absent	Absent	<50	S
French Onion Dip	3	50*	<3	<50	Absent	Absent	<50	S
Premium Vanilla Creamy yoghurt	3	36000000	10	<50	Absent	Absent	<50	М
Strawberry Low Fat Creamy								
yoghurt	3	110000000	<3	<50	Absent	Absent	<50	S
Apple and Cinnamon muesli	2	50*	<3	<50	Absent	Absent	<50	S
Rice &		00	~ 0	700	Absent	Aboont	400	<u> </u>
Cappuccino	0	50	0	50	A1 (A I	50	0
Dessert Chocolate and	2	<50	<3	<50	Absent	Absent	<50	S
Coffee Dessert	2	<50	<3	<50	Absent	Absent	<50	S
Tiramisu	2	100*	<3	<50	Absent	Absent	<50	S
Kid's, Strawberry Yoghurt	3	10000000*	<3	<50	Absent	Absent	<50	S
Grains, Banana								
and Honey yoghurt	3	1300000	<3	<50	Absent	Absent	<50	S
Chicken Sushi roll	NP	NP	20	<50	NP	NP	NP	M
Salmon Sushi roll	NP	NP	40	<50	NP	NP	NP	M
Chicken Sushi roll	NP	NP	<3	NP	NP	NP	NP	S
Salmon Sushi roll	NP	NP	<3	NP	NP	NP	NP	S
Beef Sushi roll	NP	NP	<3	NP	NP	NP	NP	S
Prawn Sushi roll	NP	NP	<3	NP	NP	NP	NP	S
Prawn Tempera Sushi roll	NP	NP	<3	NP	NP	NP	NP	S

Italic results are re-samples, * = estimate count only, NP = Not Performed, NA = Not Applicable.