

ACT HEALTH PROTECTION SERVICE

**MICROBIOLOGICAL
QUALITY OF PREPACKAGED,
REFRIGERATED READY TO EAT FOODS**



November 2009 - June 2010

Report prepared by
Deborah Denehy, Radomir Krsteski and Simon Rockliff

BACKGROUND/OBJECTIVE

The survey looked at prepackaged refrigerated ready to eat foods (PRRTE) that should be stored under refrigeration at less than five degrees celsius, such as dips, salads, sandwiches, yoghurts and desserts. PRRTE foods have become more prevalent and available in the Australian Capital Territory (ACT) through a wider range of retailers such as service stations, cafes and supermarkets. PRRTE foods by nature are potentially hazardous food. Food Standards Australia New Zealand (FSANZ) Food Standard 3.2.2 defines potentially hazardous food as “food that has to be kept at certain temperatures to minimise the growth of any pathogenic microorganism that might be present in the food or to prevent the formation of toxins in the food”. The survey of PRRTE products was undertaken for following reasons:

- to determine the bacteriological status of PRRTE food products available on the ACT market;
- to determine the compliance of these products to FSANZ Guidelines for the Microbiological Examination of Ready-to-Eat (RTE) Foods 2001;and
- to complement audits of high-risk food retailing establishments.

STANDARDS

The FSANZ RTE Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guidelines. This Table 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

Test	Microbiological Quality (colony forming units per gram (cfu/g))			
	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				
<i>Escherichia coli</i>	<3	3-100	>100	**
Pathogens				
Coagulase positive staphylococci	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴ SET +ve
<i>Bacillus cereus</i>	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
<i>Clostridium perfringens</i>	<10 ²	10 ² -10 ³	10 ³ -10 ⁴	≥10 ⁴
Salmonella spp.	not detected in 25g			detected
<i>Listeria monocytogenes</i>	not detected in 25g	detected but <10 ² #		≥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. monocytogenes* 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 November 2009 and June 2010. A total of 99 samples were collected from 35 different ACT retail outlets. The samples were randomly collected by the Health Protection Service (HPS) Public Health Officers 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 chilled, pre-packaged, ready to eat food ranging from dips to sandwiches. All of the samples were tested for the hygiene indicators SPC and *E.coli*, and the food pathogens coagulase positive *Staphylococci*, *Salmonella* species and *Listeria monocytogenes*. Eighty one samples were tested for *Clostridium perfringens* and seventy nine samples were tested for *Bacillus cereus*. Not all samples were tested for *Clostridium perfringens* and *Bacillus cereus* due to testing media availability.

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 be tested. Unsatisfactory SPC results are not re-sampled unless pathogens are also isolated.

MICROBIOLOGICAL METHOD OF ANALYSIS

- *Salmonella* species AS 1766.2.5 (modified)
- SPC AS 5013.1 - 2004
- *Bacillus cereus* AS 5013.2
- Coagulase positive *staphylococci* AS 5013.12.2
- *Escherichia coli* ISO:16649 – 2 (modified)
- *Listeria monocytogenes* AS 1766.2.16.1 (modified).
- *Clostridium perfringens* AS 5013.16 - 2006

The sample preparation for SPC, *Escherichia coli*, coagulase positive *staphylococci*, *Clostridium perfringens* and *Bacillus cereus* consisted of:

- 25g of sample being homogenised with 225mL of 0.1% peptone diluent; with subsequent serial dilutions 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. 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 µm long and 1-1.5 µm

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.

***Clostridium perfringens* enumeration:**

Overlaid pour plates of TSCNE agar using 1ml of 10⁻² dilution and 10⁻⁴ were prepared in duplicate and incubated anaerobically at 37°C/24 h. Typical presumptive *C. perfringens* colonies are black with or without precipitation surrounding the colony. Typical colonies are then confirmed using API 20A biochemical testing kit.

***Escherichia 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.

***Listeria 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/48h 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 *Listeria 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 / DISCUSSION

SPC

All samples (99) 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). One sample was assessed as being in the Level 1 criterion and was found satisfactory with a count of 100 cfu/g.

Twenty nine samples were assessed as being in the level 2 criterion. The results for these products ranged from <50 to 8.3 x 10⁷ cfu/g. One sample (3.4%) reported a marginal result and another reported an (3.4%) unsatisfactory result. High SPC for cooked products suggests that the handling or storage of these foods may have been less than optimal. No re-samples were taken of these foods as no pathogens or *E. coli* were detected at the time of testing.

A total 69 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 as low as 200 up to 6.6×10^8 cfu/g.

E.coli

Ninety nine samples were tested for *E. coli* with ninety seven samples (97.98%) satisfactory. Two samples (2.1%) contained marginal levels of *E. coli*. Mango yoghurt (83) and Premium vanilla yoghurt (10). There were no samples in the potentially hazardous range.

The two marginal samples were processed by different companies and sold at different premises. The presence of *E. coli* can indicate poor hygiene or contamination. Mango yoghurt from the same supplier was re-sampled and did not contain any *E. coli*. Other yoghurts in the same range as the premium vanilla yoghurt were re-sampled and did not contain *E. coli*.

Coagulase positive *Staphylococcus*

Ninety nine samples were analysed for coagulase positive *Staphylococcus*. All of the samples tested samples were satisfactory i.e. coagulase positive *Staphylococcus* <100 cfu/g.

Bacillus cereus

Seventy nine samples were tested for *Bacillus cereus*. Seventy seven tested samples were satisfactory i.e. *Bacillus cereus* <50 cfu/g. Two samples contained *Bacillus cereus* at marginal levels (2.1%). They were the Apple and Cinnamon Muesli at 150 cfu/g and Couscous and Roasted Vegetable Salad at 650 cfu/g. The muesli was re-sampled and no *Bacillus cereus* was found. The Couscous and Roasted Vegetable Salad was not re-sampled due to the product not being available.

Clostridium perfringens

Eighty one samples were analysed for *Clostridium perfringens*. All of the samples tested were satisfactory i.e. *Clostridium perfringens* <100 cfu/g.

Salmonella species

Salmonella spp. was not detected in any of the ninety nine samples tested. PRRTE foods should be free of *Salmonella* spp. as consumption of food containing this pathogen may result in food borne illness.

Listeria monocytogenes

Listeria monocytogenes was not detected in any of the ninety nine samples tested. The detection of *Listeria monocytogenes* in such foods indicates the food was inadequately cooked or the food was contaminated post preparation. The detection of higher levels (> 10^2 cfu/g) of *Listeria monocytogenes* in PRRTE foods indicates a failure of food handling controls and is also considered a public health risk.

CONCLUSION

The microbiological quality of the surveyed refrigerated, pre packaged, ready-to-eat foods sold in the ACT was found to be very good. All the surveyed foods reported no unsatisfactory results for pathogens or *E.coli*. Raw results of analysis are attached at

Appendix A. In conclusion, the results of this survey show a very high level of compliance with the Food Standards Australia New Zealand Guidelines for the Microbiological Examination of Ready-to-Eat Foods December 2001.

BIBLIOGRAPHY

1. Foodborne Microorganisms of Public Health Significance 2003, Australian Institute of Food Science and Technology Incorporated (AIFST).
2. Cook Chill for foodservice and manufacturing: Guidelines for safe production, storage and distribution, Brigitte Cox & Marcel Bauler, AIFST.
3. Guidelines for the microbiological examination of ready-to-eat foods FSANZ Dec 2001.

Appendix A

Assessment: S = satisfactory, M = marginal, U = unsatisfactory and * = estimate count only.

Sample	level	SPC	E.coli	Staph	Salmonella	Listeria monocytogenes	B. cereus	Cl. perfringens	Assesment
Sweet Homemade Custard Rice	2	2000000	<3	<50	Absent	Absent	NA	<50	M
Flavoured Yoghurt	3	290000000*	<3	<50	Absent	Absent	NA	<50	S
Fresh Hommus	3	48000*	<3	<50	Absent	Absent	NA	<50	S
Bean Salad	3	1100*	<3	<50	Absent	Absent		<50	S
Tomato and Basil Salad	3	1000*	<3	<50	Absent	Absent	<50	<50	S
Seafood pasta salad	3	6000*	<3	<50	Absent	Absent	50	NA	S
Creamy pasta salad	3	100000*	<3	<50	Absent	Absent	<50	NA	S
Roasted egg plant capcicum dip	3	<50	<3	<50	Absent	Absent	NA	NA	S
Sweet yoghurt	3	410000000*	<3	<50	Absent	Absent	NA	NA	S
Hummous	3	110000*	<3	<50	Absent	Absent	NA	NA	S
Rice custard	2	150*	<3	<50	Absent	Absent	NA	<50	S
Rice pudding	2	150*	<3	<50	Absent	Absent	NA	<50	S
Tabouli	3	120000	<3	<50	Absent	Absent	NA	<50	S
Coleslaw	3	320000	<3	<50	Absent	Absent		<50	S
Creamy pasta salad	3	70000*	<3	<50	Absent	Absent	<50	<50	S
Spinach Dip	3	32000000	<3	<50	Absent	Absent	NA	<50	S
Cucumber Dip	3	32000000	<3	<50	Absent	Absent	NA	<50	S
Eggplant Dip	3	54000000	<3	<50	Absent	Absent	NA	<50	S
Creamy pasta salad	3	290000000	<3	<50	Absent	Absent	<50	<50	S
Coleslaw	3	150*	<3	<50	Absent	Absent		<50	S
Hot Salami	3	1200	<3	<50	Absent	Absent	NA	<50	S
Pepperoni	3	2900	<3	<50	Absent	Absent	NA	<50	S
Creamy pasta salad	3	8600*	<3	<50	Absent	Absent	<50	<50	S
Rice pudding	2	<50	<3	<50	Absent	Absent	<50	<50	S
Chocolate bread and butter pudding	2	83000000	<3	<50	Absent	Absent	<50	<50	U
Avocado dip	3	12000000	<3	<50	Absent	Absent	<50	<50	S
Mango yoghurt	3	200*	83	<50	Absent	Absent	<50	<50	M
Spring onion cheese	3	26000000	<3	<50	Absent	Absent	<50	<50	S
Low fat yogurt	3	21000000	<3	<50	Absent	Absent	<50	<50	S
Sundried tomato	3	950	<3	<50	Absent	Absent	<50	<50	S
Le rice	2	100	<3	<50	Absent	Absent	<50	<50	S
Honeymels	2	1 000	<3	<50	Absent	Absent	<50	<50	S
Bircher muesli	3	16000000	<3	<50	Absent	Absent	<50	<50	S
Berry yoghurt	3	1500000	<3	<50	Absent	Absent	<50	<50	S
Creamy pot set passionfruit yoghurt	3	2600000	<3	<50	Absent	Absent	<50	<50	S
Rice custard	2	200000	<3	<50	Absent	Absent	<50	<50	S
Moroccan carrots	2	300	<3	<50	Absent	Absent	<50	<50	S
Rhubarb and ginger yoghurt	3	<50	<3	<50	Absent	Absent	<50	N/A	S
Hommus	3	100*	<3	<50	Absent	Absent	<50	N/A	S
Mango yoghurt	3	250*	<3	<50	Absent	Absent	<50	N/A	S
Banana & prune yoghurt	3	200*	<3	<50	Absent	Absent	<50	N/A	S
Guava yoghurt	3	450*	<3	<50	Absent	Absent	<50	N/A	S
Seafood pasta salad	3	9400	<3	<50	Absent	Absent	<50	N/A	S
Thick and fruity strawberry yoghurt	3	5000000*	<3	<50	Absent	Absent	<50	N/A	S

Yoghurt mango delight	3	390000000*	<3	<50	Absent	Absent	<50	N/A	S
Chocolate mousse	2	3000	<3	<50	Absent	Absent	<50	N/A	S
Dairy creme dessert	2	<50	<3	<50	Absent	Absent	<50	N/A	S
English leg ham	2	<50	<3	<50	Absent	Absent	<50	<50	S
Ricotta & Spinach ravioli salad	2	280000	<3	<50	Absent	Absent	<50	<50	S
Cooked and marinated mussels	1	100*	<3	<50	Absent	Absent	<50	<50	S
Organic fat free yoghurt	3	50000000	<3	<50	Absent	Absent	<50	<50	S
Herb & mayonnaise Chicken strips	2	50000*	<3	<50	Absent	Absent	<50	<50	S
Home made dark chocolate mousse	2	350000	<3	<50	Absent	Absent	<50	<50	S
Creamy potato salad	3	900	<3	<50	Absent	Absent	<50	<50	S
Mango cheese cake	2	100	<3	<50	Absent	Absent	<50	<50	S
Spring onion dip	3	<5000	<3	<50	Absent	Absent	<50	<50	S
Spring onion dip	3	460000	<3	<50	Absent	Absent	<50	<50	S
Red capsicum dip	3	1600000	<3	<50	Absent	Absent	<50	<50	S
Pasta salad	3	5000*	<3	<50	Absent	Absent	<50	<50	S
Strawberry and apple yoghurt	3	6600000*	<3	<50	Absent	Absent	<50	<50	S
Honey cinnamon yoghurt	3	50*	<3	<50	Absent	Absent	<50	<50	S
Chocolate mousse	2	200*	<3	<50	Absent	Absent	<50	<50	S
Hummous	3	290000	<3	<50	Absent	Absent	<50	<50	S
Mango and passionfruit yoghurt	3	95000000	<3	<50	Absent	Absent	<50	<50	S
Tzatziki dip	3	100*	<3	<50	Absent	Absent	<50	<50	S
German potato salad	3	2200	<3	<50	Absent	Absent	<50	<50	S
Berry fruit yoghurt	3	48000000	<3	<50	Absent	Absent	<50	<50	S
Bircher muesli apple and cinnamon	3	31000*	<3	<50	Absent	Absent	150	<50	S
Light alfresco salad	3	1400*	<3	<50	Absent	Absent	<50	<50	S
Vanilla yoghurt	3	58000000	<3	<50	Absent	Absent	50	<50	S
Rice pudding	2	<50	<3	<50	Absent	Absent	<50	<50	S
French onion dip	3	4200000	<3	<50	Absent	Absent	<50	<50	S
Pickled and fermented baby octopus	3	660000000*	<3	<50	Absent	Absent	<50	<50	S
Beetroot dip	3	14000	<3	<50	Absent	Absent	<50	<50	S
Thick and fruity passionfruit yoghurt	3	5000*	<3	<50	Absent	Absent	<50	<50	S
Berry twist yoghurt	3	1600	<3	<50	Absent	Absent	<50	<50	S
Tzatziki	3	<5000	<3	<50	Absent	Absent	<50	<50	S
Tzatziki	3	30*	<3	<50	Absent	Absent	<50	<50	S
French onion dip	2	50*	<3	<50	Absent	Absent	<50	<50	S
Vanilla creamy yoghurt	3	3600000	10	<50	Absent	Absent	<50	<50	M
Strawberry Low fat creamy yoghurt	3	110000000	<3	<50	Absent	Absent	<50	<50	S
Apple and cinnamon Bircher muesli	3	50*	<3	<50	Absent	Absent	<50	<50	S
Yogo mix choc chips	2	<50	<3	<50	Absent	Absent	<50	<50	S
Couscous and roast vegetable salad	2	200*	<3	<50	Absent	Absent	650	<50	S
Strawberry yoghurt	3	64000000	<3	<50	Absent	Absent	<50	<50	S
Fruche vanilla bean	2	100*	<3	<50	Absent	Absent	<50	<50	S

Mango and passionfruit yoghurt	3	420000000	<3	<50	Absent	Absent	<50	<50	S
Traditional chicken liver pate	2	280000	<3	<50	Absent	Absent	<50	<50	S
Traditional chicken liver pate	2	5100	<3	<50	Absent	Absent	<50	<50	S
Le rice cappacino	2	<50	<3	<50	Absent	Absent	<50	<50	S
Chocolate and coffee dessert	2	<50	<3	<50	Absent	Absent	<50	<50	S
Tiramisu	2	100*	<3	<50	Absent	Absent	<50	<50	S
Kids strawberry yoghurt	3	10000000	<3	<50	Absent	Absent	<50	<50	S
Yoghurt and grains banana and honey	3	1300000	<3	<50	Absent	Absent	<50	<50	S
Mexican guacomole	3	650*	<3	<50	Absent	Absent	<50	<50	S
White hommus, onion and garlic	3	3000000	<3	<50	Absent	Absent	<50	<50	S
Smoked salmon	2	5000*	<3	<50	Absent	Absent	<50	<50	S
Passionfruit yoghurt	3	<50	<3	<50	Absent	Absent	<50	<50	S
Greek style yoghurt with honey	3	92000000	<3	<50	Absent	Absent	<50	<50	S