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Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Compendium of Microbiological Criteria for Food

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Introduction

Microbiological criteria are established to support decision making about a food or process based on microbiological testing. Criteria can be developed and applied for different purposes across the food supply chain, with different consequences if the limits are not met.

Internationally, the Codex Alimentarius Commission (Codex) and the International Commission on Microbiological Specifications for Foods (ICMSF) have provided the lead on contemporary food safety management approaches and applying microbiological criteria. An important principle is that a microbiological criterion is established at a specified point in the food chain for a particular purpose. In general, this is to establish the safety of a food or to verify that the food safety control system or elements of it are working as intended.

The microbiological criteria used by food regulatory agencies generally include:

- **food safety criteria:** microbiological criteria that are applied to determine the safety of a food lot. Food safety criteria are included in the *Australia New Zealand Food Standards Code* (the Code) and are applied to food for sale (e.g. at any point following final product manufacture).
- **process hygiene criteria:** microbiological criteria applied to verify hygiene measures or control of process. Process hygiene criteria are included in Section 2 of this document. They are applied at a specified point in the manufacturing process.

Microbiological guidelines are also used by regulatory agencies to check that food for sale is safe and suitable and the food handling controls and hygienic practices of a food business are adequate. Guideline criteria indicate whether the microbiological status of a food product is within the normal/acceptable range and in this way are alert mechanisms to signal conformance with food safety controls. Microbiological guideline criteria for ready-to-eat foods are included in Section 1.

Food businesses may also establish process hygiene criteria and microbiological guidelines for their operations as well as setting **microbiological specifications** for raw materials and ingredients or finished products. A microbiological specification is a criterion applied as part of purchase arrangements to determine acceptability of ingredients or foods as required for ensuring product safety or quality.

This compendium brings together information on pathogens and indicator organisms significant to food safety; microbiological guideline criteria for ready-to-eat foods, and process hygiene criteria that have been established for specific food commodities.

SECTION 1

Microbiological guideline criteria for RTE foods

Background

Ready-to-eat (RTE) foods¹ are intended to be consumed without any further process by the final consumer that may eliminate or reduce pathogenic microorganisms that could be present to a safe level. They may be commodity based (e.g. dairy or meat products), but commonly include a combination of ingredients from more than one commodity group.

The safety and suitability of RTE foods should be ensured through adherence to food handling controls and good hygiene practices that prevent or minimise contamination by and growth of pathogenic microorganisms. In Australia these food safety requirements are set out in Chapters 3 and 4 of the Code. In New Zealand, the *Food Act 2014* and *Animal Products Act 1999* and associated regulations specify food handling controls. In this context, microbiological testing can be useful in checking/verifying whether food safety controls are in place and working as intended.

Purpose and scope

Food samples may be taken for a variety of surveillance and monitoring purposes. When microbiological testing of food samples is carried out it is important that relevant tests and suitable limits are applied so results are interpreted correctly and consistently.

The purpose of this section is to:

- provide information on which microbiological tests apply to RTE foods, based on their characteristics and processing factors
- outline criteria for assessment, including limits for interpreting results
- provide an indication of the type of follow-up actions to be taken in response to findings.

The reference limits provided allow an assessment of a single or multiple samples. They are not intended to be sampling plans for the acceptance/ rejection of food lots, but used for evaluating food handling controls.

The guideline criteria for RTE foods provided in this document are not intended to be used for food products for which food safety criteria have been established in the Code. Process hygiene criteria for specific foods/commodities are provided in Section 2.

Microbiological tests

As RTE foods include a wide range of products, the decision on what microbiological test to apply will depend on a number of factors:

- the type of ingredients used
- whether ingredients are cooked or raw
- the cooking or other processing involved in manufacture
- the level of handling after cooking or processing
- whether the food requires temperature control for safety (i.e. the characteristics of the food, such as pH and water activity, allow the growth of pathogenic microorganisms)
- presence and type of packaging
- shelf life.

¹ **Ready-to-eat food** is defined in Standard 3.2.2 of the Code.

Appendices 1 and 2 provide information on pathogens and indicator microorganisms/tests significant to food safety. A summary of bacterial pathogen/food associations for those microorganisms routinely tested and applicable to RTE foods is provided below.

Laboratory methods aren't specified in this section. The method used will depend on the reason for testing and factors such as speed, sensitivity, whether identification or quantification is required, as well as cost. For regulatory testing against food safety criteria in Schedule 27 of the Code, Standard 1.6.1 specifies reference methods to be used. For other testing, validated methods should be used.

Summary of bacterial pathogen/food associations

Pathogen	Associated foods	Why
<u><i>Bacillus cereus</i></u>	<p>Cooked foods such as:</p> <ul style="list-style-type: none"> • rice dishes including sushi • potato and pasta dishes • meat, vegetable and fish dishes (stews, curries etc.). 	<p>Spores are widespread in the environment and may be present on raw ingredients. The spores survive and are activated by cooking. When food is then cooled too slowly or displayed out of temperature control for extended periods, warm conditions allow for vegetative cells to grow to high levels and produce toxins.</p>
<u><i>Campylobacter spp.</i></u>	<p>Main food vehicles:</p> <ul style="list-style-type: none"> • Undercooked/improperly handled poultry • Raw meat • unpasteurised milk • contaminated water. 	<p><i>Campylobacter</i> spp. can colonise the intestinal tract of food-producing animals, such as chickens, cattle, sheep and pigs. Inadequate processing (e.g. undercooked poultry, unpasteurised milk) and cross contamination of RTE foods or food contact surfaces with raw meat and poultry can result in sufficient numbers being present in food to cause illness.</p>
<u><i>Clostridium perfringens</i></u>	<p>Cooked foods such as:</p> <ul style="list-style-type: none"> • meats, particularly rolled and large joints • meat containing products such as stews, gravies, curries and pies • vegetable dishes (curries, soups etc.) 	<p>Spores are widespread in the environment and are a part of normal intestinal flora of animals. The spores survive and are activated by cooking. Slow cooling/reheating, particularly of large volumes of food, provides warm, anaerobic conditions that allow for vegetative cells to grow to high levels that cause illness when ingested.</p>
<u><i>Listeria monocytogenes</i></u>	<p>RTE foods that can support the growth of <i>L. monocytogenes</i> and have an extended refrigerated shelf life. Foods that have been associated with outbreaks include soft cheeses, delicatessen meats, cooked chicken, smoked seafood, salads and rockmelon.</p>	<p><i>L. monocytogenes</i> is widespread in the environment and able to persist in food processing environments. RTE foods can become contaminated post processing through contamination from food-contact surfaces. <i>L. monocytogenes</i> is able to grow at refrigeration temperatures and can reach high levels in food that supports its growth.</p>
<u><i>Salmonella spp.</i></u>	<p>A wide range of foods have been implicated in outbreaks of foodborne</p>	<p><i>Salmonella</i> is widely dispersed in the environment. A primary reservoir is the intestinal tracts of vertebrates,</p>

Pathogen	Associated foods	Why
	<p>salmonellosis:</p> <ul style="list-style-type: none"> • animal products such as eggs (particularly raw or lightly cooked egg dishes), poultry, raw meat, milk and dairy products • fresh produce (such as leafy greens, seed sprouts, melons) • low moisture foods such as spices, peanut butter, chocolate and flour. 	<p>including livestock, wildlife, domestic pets, and humans. Contaminated raw foods that are eaten without further processing (such as cooking); cross contamination during food handling and poor hygiene and temperature control practices are factors contributing to food borne salmonellosis.</p>
<p><u>Shiga Toxin-producing Escherichia coli (STEC)</u></p>	<p>Foods include:</p> <ul style="list-style-type: none"> • inadequately cooked ground beef (hamburger patties) • uncooked fermented comminuted meat (e.g. salami) • raw or inadequately pasteurised dairy products (milk and cheese) • fresh produce such as leafy greens and sprouted seeds. 	<p>Ruminants, in particular cattle and sheep, are the major animal reservoir of STEC. Infected animals shed the bacteria in their faeces, resulting in contamination of the environment. Primary products (such as meat, milk and fresh produce) can be either contaminated directly by faecal material or indirectly via contaminated water or soil. STEC infection is associated with contaminated foods that are eaten without further processing or have been inadequately processed.</p>
<p><u>Staphylococcus aureus</u></p>	<p>A variety of foods, particularly those high in protein and requiring extensive handling during preparation. These can include:</p> <ul style="list-style-type: none"> • meat and meat products • poultry and egg products • milk and dairy products • cream or custard filled bakery products • sandwich fillings. 	<p>Food handlers are the main source of food contamination via direct contact (staphylococci can normally be present in people's nasal passages, throat and skin). Contamination of food can occur via hands or respiratory secretions. Time and temperature abuse of contaminated food can result in growth of <i>S. aureus</i> and production of enterotoxin in the food.</p>
<p><u>Vibrio parahaemolyticus</u></p>	<p>Fish, shellfish and crustaceans (particularly raw molluscs and crustacea) are predominantly associated with foodborne illness caused by <i>V. parahaemolyticus</i>.</p>	<p><i>V. parahaemolyticus</i> occurs in coastal and estuarine waters and is a natural contaminant of seafood. Initial levels will depend on environmental factors at harvest. Illness is associated with eating raw or lightly cooked seafood, or cooked seafood that has been cross contaminated. Inadequate refrigeration of seafood contaminated with <i>V. parahaemolyticus</i> allows growth to levels that cause illness.</p>

Interpretation of results

The tables below provide guidance on interpreting results for the microbiological examination of RTE foods for pathogenic microorganisms and for indicator microorganisms. The limits apply to foods sampled in the retail chain (i.e. food for sale at retail, food service wholesale and distribution) up to and including end of shelf life.

There are four categories of microbiological assessment defined based on the detection or level of microorganism found:

- **Satisfactory:** results are within expected microbiological levels (lower range) and present no food safety concern. No action required.
- **Marginal:** results are within expected microbiological levels but are at the upper range. Some action may be required to ensure food handling controls continue to be effective.
- **Unsatisfactory:** results are outside expected microbiological levels and indicate poor food handling practices. Further actions are required to re-establish effective food handling controls.
- **Potentially hazardous:** results exceed expected microbiological levels to a level that presents an immediate food safety concern. Further action is required to:
 - prevent affected product still available from being distributed or sold
 - determine the likely source/cause of the problem and ensure corrective actions are implemented.

Interpretation of results should also be based on knowledge of the food product and the production process. Care must be taken when interpreting results obtained in the absence of this information.

Table 1. Interpreting results for testing of pathogenic microorganisms in ready-to-eat food²

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
<u><i>Bacillus cereus</i></u> and other pathogenic <i>Bacillus</i> spp.	>10 ⁵	Potentially hazardous	Inadequate time and temperature control during cooling and subsequent storage allowing spores to germinate and multiply. The use of poor quality highly contaminated raw ingredients, such as plant based powders and spices, may also be a contributing factor. Inadequate acidification of foods using pH to control growth (e.g. acidified rice for sushi).	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. Reprocessing of product not an option due to potential for toxin formation. • Investigate and review temperature and time profiles used for the cooling and storage of cooked foods. • Identify high risk raw ingredients and consider limits for <i>B. cereus</i>. • Investigate pH and acidification process (as applicable)
	10 ³ – ≤10 ⁵	Unsatisfactory	As above.	<ul style="list-style-type: none"> • Investigate and review temperature and time profiles used for the cooling and storage of cooked foods. • Identify high risk raw ingredients and consider limits for <i>B. cereus</i>.
	10 ² – <10 ³	Marginal	Process controls not fully achieved or possible raw material contamination.	<ul style="list-style-type: none"> • Proactive investigation to ensure temperature and time profiles used for cooling and storage of cooked foods are being implemented. • Assess quality of high risk raw ingredients.
	<10 ²	Satisfactory		
<u><i>Clostridium perfringens</i></u>	>10 ⁵	Potentially hazardous	Inadequate time and temperature control during cooling, storage, processing or reheating. Slow or inadequate cooling, reheating or cooking of large production volumes a possible factor.	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. Reprocessing of product not an option due to potential for toxin formation. • Investigate and review temperature and time profiles used for the cooling and storage of cooked foods (i.e. times taken to reach required internal temperatures).

² Table 1 does not include an exhaustive list of pathogens and for some foods/circumstances, testing of other microorganisms may be appropriate. The microbiological testing applied should be appropriate to the type of food being examined and the handling it has received.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
				<ul style="list-style-type: none"> Assess capacity of business and equipment used to effectively process the volume of food handled.
	$10^3 - \leq 10^5$	Unsatisfactory	As above.	<ul style="list-style-type: none"> Investigate and review temperature and time profiles used for the cooking, cooling, storage and reheating of cooked foods (i.e. times taken to reach required internal temperatures). Assess capacity of business and equipment used to effectively process the volume of food handled.
	$10^2 - < 10^3$	Marginal	Process controls not fully achieved.	<ul style="list-style-type: none"> Proactive investigation to ensure temperature and time profiles used for cooling, processing, reheating and storage of cooked foods are being implemented.
	$< 10^2$	Satisfactory		
<u>Staphylococcus aureus</u>	$> 10^4$	Potentially hazardous	Inadequate temperature control and poor hygienic practices.	<ul style="list-style-type: none"> Product disposition action required to assess safety and determine if disposal or product recall is required. Reprocessing of product not an option due to potential for toxin formation. Food handling practices should be investigated to: <ul style="list-style-type: none"> ensure all practicable measures are being undertaken by food handlers to prevent unnecessary contact with RTE food ensure good levels of personal hygiene review temperature and time controls. Testing for enterotoxin should be considered where cases of food-borne illness are suspected.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
	$10^3 - \leq 10^4$	Unsatisfactory	As above	<ul style="list-style-type: none"> • Food handling practices should be investigated as above. • The level of <i>S. aureus</i> determined at the time of analysis may not be the highest level that occurred in the food. If cases of foodborne illness are suspected, testing for enterotoxin should be considered.
	$10^2 - <10^3$	Marginal	Hygiene and handling controls not fully achieved.	<ul style="list-style-type: none"> • Proactive investigation to ensure hygiene practices and temperature controls are effectively implemented.
	$<10^2$	Satisfactory		
<i>Listeria monocytogenes</i> ³ 1. RTE food in which growth of <i>Listeria monocytogenes</i> can occur	Detected in 25g	Potentially hazardous	<p>Post-processing or post-harvest contamination or inadequate process control.</p> <p>Higher levels in product in the marketplace may be due to poor temperature control during storage and/or distribution or inappropriate length of shelf life.</p>	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. Vulnerability of population to be considered. • An investigation should be undertaken of: <ul style="list-style-type: none"> — the raw materials used; — adequacy of cleaning and sanitising of premises and equipment, particularly of preferred sites such as drains — adequacy of construction and maintenance of premises — the effectiveness of processing controls — the adequacy of process flow. • Increased sampling, including environmental sampling.
	Not detected in 25g	Satisfactory		
2. RTE food in which growth of <i>Listeria monocytogenes</i> will not	$>10^2$	Potentially hazardous	Post-processing or post-harvest contamination or inadequate process control.	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. Vulnerability of population should be considered.

³ Schedule 27 of the Code specifies microbiological criteria for RTE food on the basis of whether growth of *L. monocytogenes* can occur or will not occur.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
occur				<ul style="list-style-type: none"> An investigation should be implemented as above.
	Detected but $\leq 10^2$	<p>Satisfactory if a listericidal process has not been applied.</p> <p>Marginal if a listericidal process has been applied.</p>	Indicates better process control required.	<ul style="list-style-type: none"> While regulatory limits are met, the presence of <i>L. monocytogenes</i> should be investigated in the case of a food that has received a listericidal process. For foods that have not, ongoing trend analysis should be used to monitor levels. Product disposition action may be required to assess safety and determine if disposal or recall is required. Vulnerability of population should be considered.
	Absent in 25g	Satisfactory		
<i>Vibrio parahaemolyticus</i>	$>10^4$	Potentially hazardous	Poor temperature control (rapid chilling and storage at $< 5^\circ\text{C}$), inadequate processing, cross contamination or high contamination levels in harvested seafood.	<ul style="list-style-type: none"> Product disposition action required to assess safety and determine if disposal or product recall is required. May need confirmation to determine whether the genetic markers of virulence are present and that the <i>V. parahaemolyticus</i> are able to cause disease. An investigation should be undertaken to assess: <ul style="list-style-type: none"> the source of raw product and potential for high levels of contamination (e.g. harvest water temperature and water salinity) the adequacy of the time and temperature controls (chilling and storage) implemented post-harvest the adequacy of the processing used (e.g. adequate cooking) likelihood of cross contamination Confirmation of identity and typing may be required where cases of food-borne illness are suspected.
	$10^2 - 10^4$	Unsatisfactory	As above	<ul style="list-style-type: none"> An investigation should be undertaken as above.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
	<3 – 10 ²	Marginal	Indication that temperature control or food handling controls are not fully achieved. It may be expected that naturally contaminated raw seafood may have low levels present (<100 cfu/g).	<ul style="list-style-type: none"> • Proactive investigation to ensure temperature and food handling controls are effectively implemented.
	<3	Satisfactory		
Shiga toxin producing Escherichia coli (STEC)	Detected in 25g	Potentially hazardous	Inadequate processing of raw products or cross contamination of raw materials and prepared foods. Poor time and temperature control is a contributing factor for multiplication.	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. • An investigation should be undertaken to assess: <ul style="list-style-type: none"> — Raw material suitability — the adequacy of processing used (e.g. adequate cooking, pH, water activity) — the adequacy of measures implemented to prevent the likelihood of cross contamination — the adequacy of time and temperature controls used. <p>Additional sampling of foods and environmental samples may be required.</p> <p>Confirmation of toxigenic strains and serotyping required where cases of foodborne illness suspected.</p>
	Not detected in 25g	Satisfactory		
Campylobacter spp.	Detected in 25g	Potentially hazardous	Inadequate processing of raw products (especially poultry and raw milk) or cross contamination of raw materials and prepared foods. The use of inadequately treated water can also be a factor.	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. • An investigation should be undertaken to assess: <ul style="list-style-type: none"> — the adequacy of processing used (e.g. adequate cooking; pasteurisation) — the adequacy of measures implemented to prevent the likelihood of cross contamination — the possibility of untreated water being used.

Hazard	Result (cfu/g)	Interpretation	Likely cause	Actions
	Not detected in 25g	Satisfactory		Ensure sample has not been frozen as results may not be accurate (<i>Campylobacter</i> levels are reduced by freezing)
<u>Salmonella spp.</u>	Detected in 25g	Potentially hazardous	Inadequate processing of raw products, cross contamination or contaminated raw materials. Poor time and temperature control is a contributing factor for multiplication.	<ul style="list-style-type: none"> • Product disposition action required to assess safety and determine if disposal or product recall is required. • An investigation should be undertaken to assess: <ul style="list-style-type: none"> — Raw material suitability — the adequacy of processing used (e.g. adequate cooking, pH, water activity) — the adequacy of measures implemented to prevent the likelihood of cross contamination — the effectiveness of cleaning and sanitising equipment (e.g. blenders, vitamisers, other processing equipment) — the adequacy of time and temperature controls used. • The adequacy of health and hygiene practices may also require investigation if an infected food handler is suspected. • Confirmation of identity, serotyping, phage typing required where cases of foodborne illness suspected.
	Not detected in 25g	Satisfactory		

Table 2. Interpreting results for testing of [indicator organisms](#) in ready-to-eat foods

Indicator	Result (cfu/g)	Interpretation	Likely cause	Actions
Enterobacteriaceae ⁴ (includes coliforms)	>10 ⁴	Unsatisfactory	For processed foods indicates that contamination has occurred post processing (cross contamination from food contact surfaces, raw products or food handlers) or there has been inadequate processing. Poor temperature time control may also be a contributing factor.	<ul style="list-style-type: none"> • Review: <ul style="list-style-type: none"> — processing controls used (such as cooking temperatures) — cleaning and sanitising practices for premises and equipment — food handler hygiene — time and temperature control. Additional food or environmental samples may be required for investigation.
	10 ² –10 ⁴	Marginal	Some cross contamination or inadequate processing indicated.	Proactive investigation to ensure processing and hygiene controls are being implemented. Results may need to be compared with other food samples from the production environment for interpretation.
	<10 ²	Satisfactory		
Escherichia coli ⁵	>10 ²	Unsatisfactory	<p>For raw and processed foods indicates potential for there to have been contamination of faecal origin from poor hygienic practices (cross contamination from food contact surfaces, raw foods or food handlers) or there has been inadequate processing.</p> <p>For RTE foods that have not been processed (e.g. fresh produce), contamination from the primary production environment should be considered.</p>	<ul style="list-style-type: none"> • Review: <ul style="list-style-type: none"> — processing controls used (such as cooking temperatures) — cleaning and sanitising practices for premises and equipment — food handler hygiene — time and temperature control — primary production controls (e.g. harvest practices, water quality, fertilizers, other inputs as appropriate). Additional food or environmental samples may be required for investigation and testing for enteric

⁴ Process hygiene criteria and associated actions for Enterobacteriaceae in specific food products are provided in Section 2.

⁵ Process hygiene criteria and associated actions for *E. coli* in specific food products are provided in Section 2.

Indicator	Result (cfu/g)	Interpretation	Likely cause	Actions
				pathogens considered if appropriate.
	3 - 10^2	Marginal	While low levels may occasionally be found in RTE food, widespread detection in several foods or areas of the food production environment suggests poor hygienic practices.	Proactive investigation to ensure processing and hygiene controls are being implemented.
	<3	Satisfactory		
<i>Listeria</i> spp. (other than <i>L. monocytogenes</i>)	> 10^2	Unsatisfactory	<p>Detection of <i>Listeria</i> spp. at this level signifies that conditions may also be favourable for <i>L. monocytogenes</i> to be present. This may be due to poor food handling controls or cross contamination.</p> <p>Higher levels may also suggest poor temperature control or inappropriate length of shelf life.</p>	<ul style="list-style-type: none"> An investigation should be undertaken of: <ul style="list-style-type: none"> the raw materials used; adequacy of cleaning and sanitising of premises and equipment adequacy of construction and maintenance of premises the effectiveness of processing controls. Additional sampling, including environmental sampling should be considered (including specific testing for <i>L. monocytogenes</i>).
	$\leq 10^2$	Marginal	Indicates that food handling controls or cross contamination may become a problem.	Proactive investigation to ensure production, processing and hygiene controls are being implemented as intended. Consider additional sampling of the environment and food products.
	Not detected in 25g	Satisfactory		

Table 3. Interpretation of results for [mesophilic aerobic bacteria](#)⁶ (MAB) colony count (at 30°C) in RTE foods

Food Category		Examples	Result (cfu/g)		
			Satisfactory	Marginal	Unsatisfactory
Category 1	Applies to foods cooked immediately before consumption	<ul style="list-style-type: none"> • Hot takeaway food such as pizza • Whole cooked bakery products such as pies, sausage rolls, quiches • ready to eat cook/chill and cook/freeze meals. 	< 10 ³	10 ³ - <10 ⁵	≥10 ⁵
Category 2	Applies to RTE foods in which all components of the foods have been cooked but there is some handling before sale or consumption.	<ul style="list-style-type: none"> • Bakery products such as cakes (without fresh cream) • Fully cooked bakery products (pies, quiches etc.) that are chilled/portioned/further handled. 	< 10 ⁴	10 ⁴ - <10 ⁶	≥10 ⁶
Category 3	Applies to RTE foods which contain some components that have been cooked and then further handled (stored, sliced or mixed) before preparation of the final food or where no cooking process has been used.	<ul style="list-style-type: none"> • Dips such as hummus, tzatziki etc. • Bakery products containing fresh cream • Sandwiches⁷ • Sliced meats • Sushi rolls 	<10 ⁶	10 ⁶ - <10 ⁷	≥10 ⁷
Category 4	Foods in level 3 either have an inherently high plate count because of the normal microbial flora present or as a result of the processing received. Includes fermented, preserved and dried food products and fresh fruit and vegetables.	<ul style="list-style-type: none"> • Fermented foods including fermented and cured meats, fermented vegetables (e.g. sauerkraut, olives), ripened cheeses, yoghurts, cultured butter, etc. • Preserved foods (pickled, marinated or salted fish or vegetables) • Dried foods (fruits, nuts, seeds, herbs, spices, dried fish/meat) • Whole fresh fruits and vegetables and foods containing these e.g. salads; sandwiches containing salad or vegetable ingredients. 	N/A	N/A	N/A

⁶ Also known as standard plate count (SPC), aerobic plate count (APC), total viable count (TVC).

⁷ For sandwiches that contain salad or vegetable ingredients, higher counts may be attributed to the microbial flora associated with those ingredients.

SECTION 2

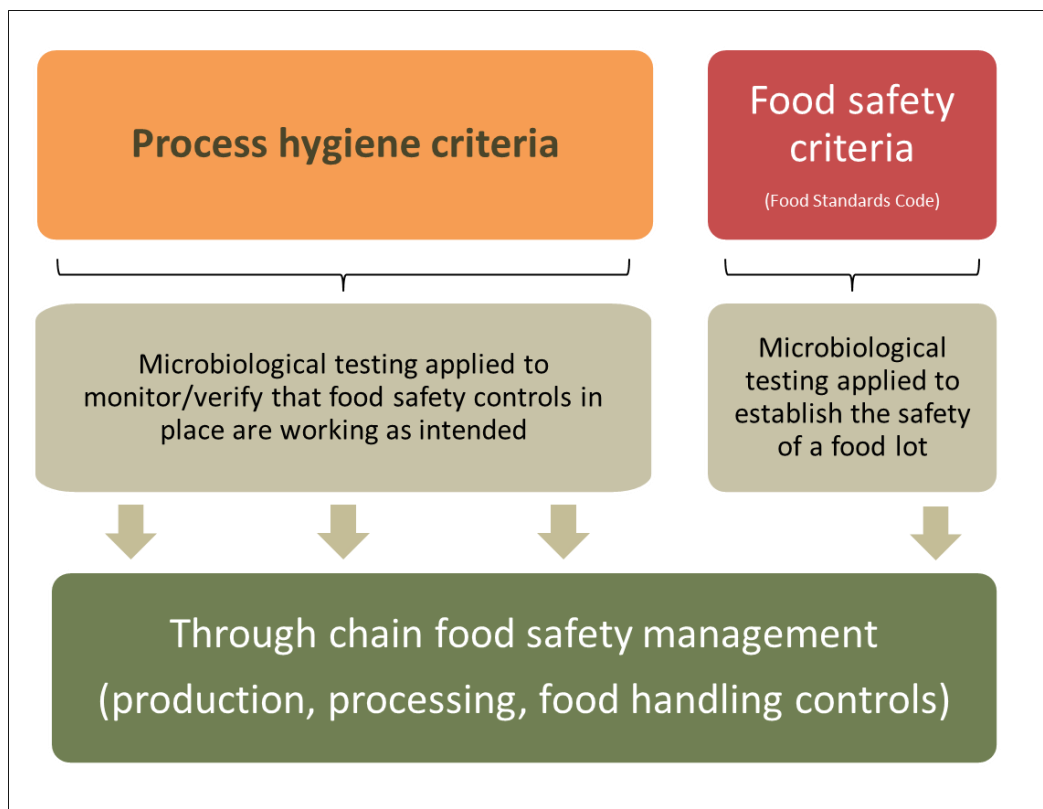
Process hygiene criteria

Food safety is best ensured by implementing food hygiene controls at each stage of food handling throughout the food chain. In Australia these food safety requirements are set out in Chapters 3 and 4 of the Code. In New Zealand, the *Food Act 2014* and *Animal Products Act 1999* and associated regulations specify food handling controls and may include process hygiene criteria as guidance.

Microbiological testing can be a useful tool to support through chain control measures. Microbiological criteria may be established to examine ingredients, in-process and environmental samples and food products that may be collected at different points in the food system, from primary producers, through production and retail.

Process hygiene criteria are microbiological criteria applied to verify hygiene measures or control of process. They can be applied at various stages throughout the production process to indicate whether the food safety controls in place are working as intended. Non-conformance with a process hygiene criterion should result in corrective actions to adjust the process, as appropriate, and ensure ongoing control of production.

The process hygiene criteria provided in Section 2 have been developed by regulatory agencies in consultation with relevant food industry sectors. They apply to process control testing programs. For some foods, food safety criteria have also been established in the Code. Food safety criteria can be applied by relevant authorities to sample and test the safety of a food lot available for sale. Applying testing programs to detect loss of control before a food safety limit is exceeded, however, provides a proactive approach to ensuring safe and suitable food.



2.1 Powdered infant formula products

Safe production of powdered infant formula products⁸ depends on maintaining a high level of hygiene control to prevent entry and establishment of pathogens such as *Salmonella* and *Cronobacter*⁹ in processing areas. The Codex *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children* (CAC/RCP 66 – 2008) provides guidance on the hygienic manufacture of powdered infant formulae and on the subsequent hygienic preparation, handling and use of reconstituted formula products.

Microbiological food safety criteria for *Salmonella* and *Cronobacter* in powdered infant formula and powdered follow on formula are specified in Schedule 27 in the Code. These criteria apply for regulatory testing purposes. To meet the stringency of the sampling plans specified for these pathogens it would be expected that infant formula manufacturers use routine microbiological sampling and testing as part of monitoring and verification of the food safety control system they have in place. This may include testing ingredients, the processing environment, in process samples and final product testing.

Process hygiene criteria

Testing for Enterobacteriaceae and Mesophilic Aerobic Bacteria is useful to verify that the hygiene measures in place in a manufacturing facility are working as intended. This provides assurance that the potential for pathogens such as *Salmonella* and *Cronobacter* to be in the processing environment and to cross-contaminate infant formula products is being controlled.

Process hygiene criteria for Enterobacteriaceae and Mesophilic Aerobic Bacteria in powdered infant formula products are provided below.

Powdered Infant formula products	(n)	(c)	(m)	(M)
Mesophilic Aerobic Bacteria	5	2	500/g	5000/g
Enterobacteriaceae ¹⁰	10	2	0/10g	-

n = number of sample units

c = the number of sample units allowed to exceed m

m = the acceptable microbiological limit

M = the limit which must not be exceeded

These process hygiene criteria apply to the finished product or at any other point in manufacture that provides the information necessary to verify process control. They are intended to be used by the manufacturer as a means of ongoing assessment of their hygiene programs.

Mesophilic aerobic bacteria counts provide useful indications on the hygienic status of wet processing steps. A trend in counts above the recommended limits may indicate a build-up of

⁸ Infant formula products is defined in Standard 1.1.2 of the Code

⁹ Referred to as *Enterobacter sakazakii* prior to 2008.

¹⁰ Codex proposed a 2 class sampling plan for Enterobacteriaceae on the basis that a 3 class sampling plan would not be practical analytically given the low levels of Enterobacteriaceae that occur when stringent hygiene conditions are maintained. This criterion assumes that:

- the product is sufficiently homogenous so that high level contaminations will fail (more than two samples would exceed “m”)
- in practice, positives would not normally be found if strict hygiene measures are in place. If occasional positives are found, the manufacturer would take appropriate actions.

bacteria in equipment such as evaporators or contamination due to leaks in plate-heat exchangers (Codex, 2008). These limits shouldn't be applied to powdered infant formula products that contain lactic acid producing microorganisms.

Failure to consistently meet criteria for Enterobacteriaceae may be a trigger to examine environmental and process hygiene controls and to evaluate product safety through increased sampling of final product for *Cronobacter* and *Salmonella*. Finding 1 or 2 positives should indicate a trend toward potential loss of process control. Finding 3 or more positives should signal loss of process control and appropriate actions should be taken including:

- Evaluation of product safety through increased sampling of final product for *Cronobacter* and *Salmonella* before release of the product
- Evaluation of environmental and process hygiene controls to confirm they are suitable and are able to maintain hygiene control continuously before production is resumed.

The reference methods for Mesophilic aerobic bacteria and Enterobacteriaceae should be the most recent Australian Standard (AS 5013 series) or International Organization for Standardization (ISO) methods, or other validated methods that provide equivalent sensitivity, reproducibility and reliability.

Microbiological specifications

Critical ingredients that do not undergo a heat treatment during processing (e.g. dry mix ingredients) need to be able to meet microbiological requirements for the final product. The ICMSF (2011) suggest sampling and testing for *Salmonella* and *Cronobacter*, as well as Enterobacteriaceae, should be considered either for acceptance or as monitoring, depending on the confidence level in the supplier.

Additional considerations

FAO/WHO Expert Consultations (2004, 2006) categorised *Staphylococcus aureus* and *Bacillus cereus* as "Microorganisms for which causality with illness is less plausible or not yet demonstrated" (Category C). It is generally accepted that low levels (<100 cfu/g) of these microorganisms may be present in powdered infant formula products and should be managed and monitored by the manufacturer as appropriate.

References

Codex (2008) *Code of hygienic practice for powdered infant formulae for infants and young children* (CAC/RCP 66 – 2008), http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1 accessed 28 July 2015.

International Commission on Microbiological Specifications for Foods (2011) *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance*. Springer, New York.

FAO/WHO (2006) *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting Report

FAO/WHO (2004) *Enterobacter sakazakii* and microorganisms in powdered infant formula: Meeting Report

2.2 Raw chicken meat

The main microbiological hazards associated with raw poultry meat are contamination with *Salmonella* and *Campylobacter* (FSANZ, 2005). An effective food safety management system includes control points throughout production and processing to control these hazards.

Microbiological testing is one of a number of indicators of effective process control in production areas and during processing of poultry meat. It cannot be used as a sole measure of compliance or in isolation from other measures, rather as an indicator of an effective food safety control system operating within a business.

Government regulatory bodies may also utilise information collected from verification points by the business (including microbiological testing of carcasses) to support assessments of processing establishments. Information collected by businesses may assist regulators to verify the overall performance of the business' food safety system.

These microbiological targets should be used within the context of through chain controls to¹¹:

- support and to verify effective application of process controls
- provide feedback to food business operators on microbiological levels which should be achieved when applying best practices
- assist in identifying situations (products and processes) requiring investigative action and/or control action

Process hygiene criteria

Campylobacter

A microbiological target for *Campylobacter* of **<10,000 CFU/ whole chicken carcass**¹² at the end of processing (after final chill and just prior to dispatch) assists in verifying that the whole process is under control.

If processors meet the designated target this verifies that their process is maintaining suitable control. Corrective actions to be taken when the criteria are not met should include review of process controls, including:

- for birds prior to entering the slaughter facility
- following evisceration and prior to birds entering the washing process
- for the carcass decontamination process
- for chilling of poultry meat carcasses

Further detail is provided at Attachment 1.

Noting that 5,000 *Campylobacter* organisms per carcass could be considered to be sufficient to cause a risk of cross contamination to ready-to-eat foods in the kitchen environment, it is recommended that a technique to count down to a lower level is used as this can be readily

¹¹ This guidance should be read in conjunction with the Australian Standard AS 4465-2006 *Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption* and Appendix A to provide additional and specific guidance with regard to *Salmonella* and *Campylobacter* across the industry

¹² Please note that based on available data these targets are only for chicken meat at this stage

achieved in the processing plant and can demonstrate good process control (see Methods of analysis).

Salmonella

A microbiological target for *Salmonella* has not been proposed however, if present, serotypes should be identified. Specific *Salmonella* serotypes of public health or industry significance (i.e. *Salmonella* Typhimurium or *Salmonella* Enteritidis) must be notified immediately where required to the relevant authority to ensure appropriate controls are applied. The controls through the process which reduce counts of *Campylobacter* are the same which can control *Salmonella*.

Identity of *Salmonella* types on carcasses is important as an assessment of control measures throughout the food chain and so requires an examination of risk and control of hazards as stated in the Primary Production and Processing Standard for Poultry Meat (Standard 4.2.2). There are many ways that a company can demonstrate risk assessment and may include targeted microbiological surveillance and may necessitate investigation and corrective actions further back up the food chain, such as, but not limited to:

- Breeder farms
- Hatcheries
- Feed production
- Transport
- Broiler farms
- Livehaul equipment and transport
- Processing plant equipment cleaning and maintenance

Verification points

The performance of through-chain system controls within poultry processing cannot be fully verified through the isolated application of microbiological end-point testing. In order to do so, information should be gathered that relates to processing performance at designated verification points through the entire chain, including live bird receipt, evisceration, carcass decontamination and chilling. Achieving performance targets at each of these verification points, may provide evidence to demonstrate effective operation of the process controls in place. Ideally, information collected should form part of the periodic validation for each business' food safety management system as part of their compliance arrangement in consultation with the regulatory authority.

In order to effectively monitor verification points (Attachment 1), businesses should assess their individual circumstances and develop an appropriate monitoring regime including sampling size and monitoring frequency that accounts for a number of factors within the business. These factors may include (but not be limited to) the size of the business, the quantum of productive output, how the product is presented to the end user and the risks associated with the scale of activities being conducted. This should be done in consultation with the regulator. Additionally, all processors should maintain records to demonstrate process control (including details of appropriate corrective actions if out of specification).

Regular monitoring of all verification points enables businesses to make timely assessments of food safety system performance, which may be then further verified by an associated microbiological test. A microbiological testing programme needs to be developed by each processor to regularly demonstrate that process control is achieving suitable management of microbiological contamination. This should be completed at a frequency which builds confidence and demonstrates that the management of process control is sufficient to minimise the risks. As noted above, microbiological testing is not a sole determinant of an

effective food safety management system. Therefore, in developing a monitoring plan, each food business should consider what appropriate corrective actions in each instance may be, and when they are to be taken if monitoring activities indicate that applied process controls may not be operating effectively.

Method of analysis

Three methods of increasing sensitivity are provided. At a minimum, the method to be used should be the most recent Australian Standard (AS 5013 series) or International Organization for Standardization (ISO) method, or other validated method that provides equivalent sensitivity, reproducibility and reliability.

1. AS.5013.6:2015 Food microbiology. Method 6: Examination for specific organisms – *Campylobacter* will achieve a limit of detection of 5,000 *Campylobacter* organisms per carcass when rinsing whole carcasses with 500ml of rinsate and plating out one ml of rinsate.
2. The New Zealand Ministry of Primary Industries has developed a technique which is used in the poultry industry whereby the carcasses are rinsed with 400ml of rinsate and 2ml of rinsate are plated on to 6 plates. This gives a limit of detection of 200 *Campylobacter* organisms (Lee *et al.* 2014). The modified *Campylobacter* method below is a further adaptation of this technique which can be used for carcasses or portions. Other validated methods are also appropriate such as a miniaturized most probable number method as published by Chenu *et al.* (2013).
3. Modified *Campylobacter* method to lower the LOD (limit of detection) to 100 CFUs (colony forming units) per carcass:
 - Each carcass is rinsed with 200ml of sterile buffered peptone water for 2 minutes. 2ml of rinse fluid is inoculated over eight (8) *Campylobacter* Blood Free Agar plates (250µl per plate).
 - A 100µl aliquot of rinse is plated onto a ninth plate for higher concentrations of organisms. The plates are placed in sealed containers with atmosphere generating sachets (CampyGen™, Oxoid) and incubated at $42 \pm 0.5^\circ\text{C}$ for 48 ± 2 hours.
 - After incubation, up to five representative colonies are selected from across the eight plates. The selected colonies are confirmed as *Campylobacter* by oxidase activity and latex agglutination test, *Campylobacter* Dryspot *Campylobacter* Test™ (Oxoid).

Note: A 200mL rinse was used to achieve greater sensitivity and 8 x 250µL inoculums used for greater precision.

The number of *Campylobacter* CFU per sample is calculated by adding up the number of confirmed colonies counted on the 8 plates:

(plate 1 + plate 2 + plate 3 + plate 4 + plate 5 + plate 6 + plate 7 + plate 8) x 200ml/2ml = number of *Campylobacter* organisms per poultry sample.

For duplicate plates of higher dilutions:

CFU per sample = *(number colonies confirmed as *Campylobacter*/n) x count characteristic *Campylobacter* morphology colonies (plate 1 + plate 2)/2 x 200ml/0.1ml x 1/dilution = number of *Campylobacter* organisms/poultry carcass sample

where:

n = number or characteristic colonies examined (usually 5 unless there are less than 5 characteristic colonies altogether).

* Usually five/five if the first colony of five is confirmed as positive. Will be reported as a proportion of five, if the remaining colonies are required to be confirmed e.g. three/five.

References

Chenu, J., Pavic, A., Cox, J. (2013) A novel miniaturized most probable number method for the enumeration of *Campylobacter* spp. from poultry-associated matrices. *Journal of Microbiological Methods* 93:12-19

Lee, J., Castle, M., Duncan, G., Hathaway, S., van der Logt, P., Wagener, S., LassoCruz, A., Gichia, M., Tebwe, T., Silva, U. (2014) Example of a microbiological criterion (MC) for verifying the performance of a food safety control system: *Campylobacter* Performance Target at end of processing of broiler chickens. *Food Control* 58: 23-28
<http://dx.doi.org/10.1016/j.foodcont.2014.07.012>.

FSANZ (2005) Scientific assessment of the public health and safety of poultry meat in Australia. Food Standards Australia New Zealand.
<http://www.foodstandards.gov.au/code/primaryproduction/poultry/pages/default.as>

System Assessment – Control Point Checklist

Verification Point	1 – Control Point: Live Bird Receipt	2 – Control Point: Evisceration	3 - Control Point: Carcase Decontamination	4 – Control Point: Storage, Further Processing and Distribution
Performance target	8 - 12 hours off feed	No unacceptable carcasses enter the carcase wash	>5 ppm FAC pH 5 – 7 ORP >650mV Note : Upper limits need to ensure compliance with S18-7 in Food Standards Code.	Poultry meat carcasses chilled to <7°C within 6 hours of stunning, ≤5°C within 12 hours and maintained at ≤5°C
Outcome	<p>Only birds that are fit for human consumption are to be processed; no feed in the crop; minimal gut spillage.</p> <p>This control point is designated to identify and control hazards prior to entering the slaughter facility. It applies to all stock prior to processing and can be applied to all processing establishments regardless of size.</p> <p>Farms are required to remove feed (but not water) from flocks prior to pick-up and transport, and provide evidence (i.e. a declaration) to the processor that feed withdrawal and any veterinary withholding periods have been met and birds have been examined to ensure suitability for</p>	<p>Each carcase is subject to inspection and appropriate disposition</p> <p>This control point is designated to identify and control hazards associated with evisceration. It applies to all processing establishments performing manual or mechanical evisceration.</p> <p>The purpose of this control point is to establish a system that identifies contamination or any other condition that makes a carcase otherwise unacceptable and manages it at the earliest possible opportunity. This reduces pressure on the control points further along the processing chain and minimises contaminants entering the washing process.</p>	<p>Each carcase is subject to an overall reduction in microbial load through the wash/chill process.</p> <p>This control point is designated to control hazards associated with microbiological contamination of carcasses. It applies to all processing establishments washing carcasses after manual or mechanical evisceration using either a spin wash/chill system or other immersion or washing process.</p> <p>The purpose of this control point is to establish a system that achieves a consistent validated reduction in overall microbial load on carcasses to improve food safety in regards to pathogens of concern (i.e. <i>Campylobacter</i> spp. and <i>Salmonella</i></p>	<p>Each carcase is subject to a chilling process that supports an overall reduction in microbial load through processing.</p> <p>This control point is designated to control hazards associated with microbiological growth on poultry carcasses.</p> <p>The purpose of this control point is to establish a system that achieves a consistent validated reduction of temperature to meet the requirements of AS4465 and minimise microbiological growth to maintain minimal pathogenic loads on carcasses and preserve product quality.</p>

	<p>slaughter for human consumption.</p> <p>Significant evidence exists that demonstrates that poultry presented for slaughter with less than 8 hours feed withdrawal are subject to greater levels of contamination by spilled ingesta. Similarly, flocks that are off feed greater than approximately 12 hours are susceptible to bile production and can suffer from increased rates of intestinal tearing during evisceration due to weakening of the gastrointestinal tract due to sloughing of cells. Therefore, the target between 8-12 hours, should be considered in order to meet the outcome without compromising further processing.</p>		<p>spp.) in addition to improving product quality. The microbiological verification targets are indicative of the effective operation of the carcass decontamination process.</p>	
When	<p>Prior to birds being presented for slaughter at abattoir.</p>	<p>At point of final inspection, prior to birds entering the washing process (i.e. the end of the evisceration) during processing operations.</p>	<p>Wash water is measured at the point of overflow from the washing system (or a designated point within decontamination system) at defined intervals during processing.</p>	<p>Temperature of carcass and monitored at the end of processing (after final chill and just prior to dispatch).</p>
What	<p>All birds must be kept off feed for a sufficient period of between 8 -12 hours prior to slaughter to ensure crops are empty.</p> <p>Birds are to remain on water until pickup commences (minimum of 2 hours before pick-up).</p>	<p>All carcasses must be subject to a visual inspection from a suitably trained person, prior to entering the wash.</p> <p>Unacceptable birds must be removed from the processing line and managed separately.</p>	<p>The operation of the carcass decontamination system (e.g. spin wash/chiller, inside/outside washer) must conform to a set of validated operating conditions that demonstrates a reduction in microbiological contamination to ensure effectiveness.</p>	<p>Carcasses must be chilled to a surface temperature of 7°C or less within 6 hours of stunning and further reduced to a core temperature <5°C within 12 hours of stunning.</p> <p>Edible offal must be chilled to <5°C within 1 hour.</p>

	Ante-mortem inspection of poultry prior to slaughter by a suitably qualified person.		Target operational requirements are >5 ppm free available chlorine (F.A.C.) concentration and pH between 5 and 7. Alternative operational limits (e.g. for peracetic acid or chlorine dioxide) may be applied if sufficient validation information is provided.	Frozen product reduced to -15°C or less within 96 hours of stunning. Core temperatures of product maintained at <5°C through distribution.
How	<p>Ensure birds sourced from approved suppliers/accredited farms.</p> <p>Identification and removal of unhealthy or diseased poultry.</p> <p>Presentation of grower declaration (i.e. evidence that birds are free from chemical residues, time off feed parameters have been met etc.).</p> <p>Demonstration by the company that withholding periods for in-feed medication /treatments are met.</p> <p>Physical verification by crop check.</p>	<p>Ensure complete evisceration to minimise carcass contamination (operation of plant and performance of personnel as appropriate).</p> <p>At every processing break, and at least once per shift, evisceration machinery is disengaged from the line and all intestines and faeces are removed from the operating parts of the machine.</p> <p>Identification and removal of unacceptable carcasses from the process.</p> <p>Appropriate management system for unacceptable carcasses (e.g. re-work process).</p> <p>Verification through monitoring of evisceration efficiency at final inspection point (e.g. 100-bird assessment).</p>	<p>Collect and analyse samples of wash water from overflow point (or at most contaminated point during processing) and record pH, free available chlorine (F.A.C.) concentration and/or ORP to ensure that minimal performance targets are met and demonstrate effective decontamination of carcasses.</p> <p>Ensure continuous overflow and replenishment of active chemical to maintain effective and sanitary operational conditions.</p> <p>Verification through microbiological testing of final product: Target levels of <10 000 CFU/carcass <i>Campylobacter</i> spp..</p>	<p>Monitor temperature of chiller water during carcass/offal chilling.</p> <p>Monitor time and temperature of carcasses at exit of chiller and during post-chill processing via calibrated deep muscle probe thermometer.</p> <p>Monitor operational air temperature of chillers/freezers.</p> <p>Monitor temperature of chilled/frozen product during storage, at dispatch and through the distribution chain (e.g. data logger).</p>

<p>Why</p>	<p>Ensure that only suitable birds are presented for processing.</p> <p>Minimise contamination of carcasses during processing due to spilled intestinal contents or bile staining (intestinal tearing). Appropriate animal welfare outcomes.</p>	<p>Minimise the contamination of carcasses and ensure the effective operation of evisceration machinery.</p> <p>Ensure that condemned and inedible carcasses aren't entering the washing process. Minimise contamination of wash water and improve reduction of microbial load.</p> <p>Enable timely corrective actions to be implemented in order to minimise the amount of product affected.</p>	<p>Achieve consistent sufficient reduction of microbiological hazards through-chain and minimise microbiological contamination of carcasses.</p> <p>Enable sufficient process control for microbiological hazards to ensure that the wash/chill system operates effectively and does not increase microbiological loads on carcasses.</p> <p>Enable timely responses to be implemented in the event of non-conformance to ensure adequate process control is maintained and minimal product is affected.</p>	<p>Control hazards associated with the growth of microbiological organisms associated with final product carcasses to maintain acceptability of carcasses and shelf life.</p> <p>Enable sufficient process control during further processing and handling of product.</p> <p>Enable timely responses to be implemented in the event of non-conformance to minimise the amount of product affected.</p>
<p>Corrective action</p>	<p>Withhold from slaughter (e.g. feed detected in crop/<8hrs feed withdrawal; chemical residue suspected).</p> <p>Removal and humane slaughter of injured/diseased birds (disposal).</p> <p>Removal of dead birds (disposal).</p> <p>Isolation of suspect/diseased flocks.</p> <p>Notify relevant authority when required.</p>	<p>Adjust equipment to allow for correct operation appropriate to carcase size. Identify and re-train relevant operational staff.</p> <p>Isolate and re-work affected product within appropriate timeframes.</p> <p>Adjust/service/repair equipment to manufacturer's specification.</p> <p>Halt killing process and clean and sanitise evisceration equipment prior to restarting</p> <p>Notify relevant authority when required.</p>	<p>Ensure correct operation of spin chiller (e.g. overflow) and adjust sanitiser and/or pH levels to return to within operational parameters.</p> <p>Adjust automatic dosing equipment.</p> <p>Identify and re-train relevant operational staff.</p> <p>Notify relevant authority when required.</p>	<p>Adjust operational temperature of chilling system (e.g. add ice to spin chiller). Seal cold storage rooms to maintain temperature and monitor via data logging.</p> <p>Halt further stunning/killing process and hold in-process carcasses in chilling system.</p> <p>Identify alternative storage arrangements.</p> <p>Isolate/freeze/test-and-hold potentially affected product.</p> <p>Condemn affected product where temperature exceeds 5°C for a</p>

				<p>period of time that may compromise the wholesomeness of the product, and the product isn't compromised to the point where remedial processing wouldn't ensure the product is still acceptable for human consumption.</p> <p>Notify relevant authority when required.</p>
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APPENDIX 1 Pathogens

A number of pathogenic microorganisms can cause foodborne illness including bacteria, viruses and parasites. The significance of these agents to specific food products varies, depending on their ecology, survival and growth characteristics. Knowledge of these characteristics and typical food pathogen associations is useful to inform the choice of test(s) when microbiological examination of foods is undertaken. A snapshot of relevant information on pathogenic microorganisms commonly associated with foodborne illness is provided below. This material is not intended to be comprehensive - more detailed information is available in the other resources listed.

***Bacillus cereus* & other *Bacillus* spp.**

Description

This is a diverse group of spore forming bacterium commonly found in the environment (e.g. soil and vegetation). The spores are able to survive harsh environments including normal cooking temperatures.

Two types of foodborne illness are associated with *B. cereus* – emetic (vomiting) and diarrhoeal. The emetic syndrome is caused by ingesting heat stable pre-formed emetic toxin produced in the food during active growth of the bacteria. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine following ingestion of large numbers of the bacteria.

Foodborne illness is generally associated with high bacteria levels (greater than 10^5 cfu/g) in implicated foods. Onset of illness is fairly rapid (1- 5 hours for emetic syndrome; 8-16 hours for diarrhoeal) and symptoms generally mild and short-lived.

Other species of *Bacillus* that are associated with food-borne illness are from the *Bacillus subtilis* group (including *B. subtilis*, *B. licheniformis* and *B. pumilis*). Symptoms of illness and causative factors are similar to *B. cereus*.

Not all strains are associated with illness.

Associated foods

Given its distribution in the environment, low level contamination of many food commodities with *B. cereus* spores should be expected. When these foods are cooked vegetative cells are destroyed, however spores can survive and be activated. *B. cereus* is then able to multiply if the characteristics of the food (pH, water activity etc.) allow growth and the food is not kept under temperature control.

Foods associated with the emetic syndrome are predominantly rice dishes, though other starchy foods (potato and pasta dishes) may be involved.

There are a wide range of foods associated with diarrhoeal-type food poisoning including meat, vegetable and fish dishes, particularly those incorporating spices (spices may carry a high load of *Bacillus* spp. spores).

Slow cooling and storage of large amounts of cooked foods at temperatures between 10 - 50°C

favour *B. cereus* growth.

Control Measures

B. cereus associated with emetic toxin production are mesophilic (optimal growth temperature is 30 to 40°C). To control growth and toxin production, cooked foods should be either:

- cooled rapidly and stored at 5°C or below
- held warm at 60 °C or above
- displayed and handled according to the '2hour/ 4 hour rule' (see Appendix 1 of [Safe Food Australia](#)).

Other growth characteristics

- pH

Acid tolerance varies between strains of *B. cereus*. In general, enterotoxin is produced in the range pH 5.0 – 9.0 and the growth range is pH 4.5 – 9.0.

Growth of *B. cereus* is controlled when foods are acidified to pH <4.6.

- water activity

The minimum water activity for growth is 0.93 (where temperature and pH are optimum for growth). The maximum salt concentration for *B. cereus* for growth is in the range 7 –7.5%.

Further information on preservatives and other factors that control growth and toxin production can be found in ICMSF (1996).

Resources

[FDA \(2012\) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 93–96.](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2013\) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.](#)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

Jenson I, Moir CJ (2003) *Bacillus cereus* and other *Bacillus* species. Ch 14 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 445-478

[New Zealand Ministry of Primary Industries \(2015\) Bacillus cereus Microbial Pathogen Data Sheet.](#)

http://www.foodsafety.govt.nz/elibrary/industry/Bacillus_Cereus-Spore_Forming.pdf

Campylobacter spp.

Description

Campylobacter spp. are Gram-negative, non-spore forming bacteria, generally motile with an S-shape morphology.

C. jejuni and *C. coli* are the species primarily associated with campylobacteriosis, the gastrointestinal disease caused by *Campylobacter*. *C. jejuni* accounts for most cases of human illness.

Many domestic and wild animals such as cattle, sheep, poultry, dogs, wild birds and rodents, carry *C. jejuni* as part of their normal intestinal flora and shed the organism in their faeces. *Campylobacter* spp. are transmitted to humans predominantly through the consumption of contaminated food or water or through direct contact with infected animals. Most cases are sporadic.

Infection by *Campylobacter* spp. has been associated with ingestion of as few as 100 cells. The incubation period before onset of disease is usually 2–5 days, with illness generally lasting for 2–10 days. The major symptoms include fever, diarrhoea (sometimes bloody), abdominal cramps, headache, nausea and vomiting.

A distinctive feature of *Campylobacter* infection is the severity of abdominal pain which may become sufficiently intense to mimic acute appendicitis. As a result of infection, a small percentage of people develop secondary conditions such as reactive arthritis or Guillain-Barré syndrome.

Campylobacter spp. are microaerophilic (growing best at 5-6% O₂) and require special incubation conditions for cell isolation and growth.

Associated foods

The major food sources linked to campylobacteriosis are:

- inadequately handled or undercooked poultry and poultry products such as livers
- raw milk
- contaminated water.

Control Measures

Control measures include:

- avoiding cross contamination of raw poultry and meats to RTE foods and food contact surfaces
- thorough cooking of poultry and poultry products
- pasteurisation of milk
- only consuming/using water that has been treated (potable).

Given poultry meat is a primary source of *Campylobacter* spp., contamination levels should be minimised through appropriate controls during primary production and processing.

Growth and survival characteristics

- Temperature

C. jejuni and *C. coli* are thermophiles, growing optimally at 42°C with a growth range of 30 – 45°C. *Campylobacter* spp. are unable to grow in foods (due to the levels of oxygen) but can survive refrigerated conditions. They are easily inactivated by heating such as applied during cooking or pasteurisation.

- Water activity

Campylobacter spp. are sensitive to drying and do not survive long in dry conditions (minimum water activity for growth is 0.987).

Resources

ICMSF (1996) *Microorganisms in Foods 5: Microbiological specifications of food pathogens*. Chapman & Hall, London.

[FDA \(2012\) *Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.14-17.*](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2013\) *Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.*](#)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

[New Zealand Ministry of Primary Industries \(2001\) *Campylobacter Microbial Pathogen Data Sheet*](#)

http://www.foodsafety.govt.nz/elibrary/industry/Campylobacter-Organism_Causes.pdf

Wallace, B (2003) *Campylobacter*. Ch 10 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p359 - 380

Clostridium perfringens

Description

Clostridium perfringens is an anaerobic (or aerotolerant) spore forming bacilli that is widely distributed in the environment, with spores persisting in soils. *C. perfringens* is a part of the normal intestinal flora of humans and other animals.

Spores are able to survive normal cooking temperatures and can germinate and multiply if warm conditions prevail. Depending on the temperature and food matrix, *C. perfringens* can have a doubling time of < 10 minutes.

Illness is caused by ingestion of a large number (>10⁶) of vegetative cells that multiply and sporulate in the lower small intestine, producing an enterotoxin which causes profuse diarrhoea and abdominal cramps about 16 hours after consumption. Gastrointestinal illness is generally mild and self-limiting.

There are many strains of *C. perfringens*, not all of them producing enterotoxin. *C. perfringens* enterotoxin (CPE) is most commonly produced by type A strains.

Associated foods

Because of its widespread distribution, spores of *C. perfringens* may be present in various animal or plant food products (such as spices).

Food borne illness associated with *C. perfringens* is almost always associated with temperature abuse of cooked foods such as meats (mainly beef and poultry) and meat-containing products (e.g. gravies, stews and curries), though vegetable dishes have also been implicated in outbreaks.

C. perfringens food poisoning primarily occurs when large volumes of food are prepared and are cooled too slowly or kept at ambient temperature, so that the food is kept warm for extended periods of time. The centre of a mass of cooked food provides an anaerobic environment that allows for the growth of *C. perfringens* at these warm temperatures.

Control Measures

The primary control for *C. perfringens* in ready-to-eat foods is maintaining temperatures that prevent multiplication of vegetative cells in cooked foods.

The optimum growth temperature for *C. perfringens* is generally between 43°C to 47°C. Because of its fast doubling time, cooked foods prepared in advance need to be cooled rapidly to limit the time at these temperatures. Clause 7(3) of Standard 3.2.2 of the Code (Australia only**) specifies cooling requirements for cooked potentially hazardous foods which require cooling from 60°C to 21°C to be achieved within 2 hours*. Cooling from 21°C to 5°C should occur within a further 4 hours. Once cooled, cooked foods should be stored at 5°C or below.

Reheating previously cooked foods also needs to be rapid to minimise the time the food is kept at optimal growth temperatures. Reheating to above 70°C will kill vegetative cells of *C. perfringens* present.

pH and water activity

The growth of *C. perfringens* is inhibited below pH 5.5 and the minimum water activity for growth is 0.97.

* Standard 3.2.2 provides for an alternative cooling process to be used where it can be demonstrated that the microbiological safety of the food will not be adversely affected.

**Similar time-temperature requirements for the cooling of foods are also included in New Zealand legislation.

Resources

Bates, J & Bodnaruk, P (2003) *Clostridium perfringens*. Ch 15 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p479-542

[FDA \(2012\) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p.14-17.](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2001\) Safe Food Australia. 2nd ed, Food Standards Australia New Zealand Canberra.](#)

<http://www.foodstandards.gov.au/publications/Pages/safefoodaustralia2nd519.aspx>

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

[New Zealand Ministry of Primary Industries \(2010\) Clostridium perfringens Microbial Pathogen Data Sheet.](#)

http://www.foodsafety.govt.nz/elibrary/industry/Clostridium_Perfringens-Associated_With.htm

Listeria monocytogenes

Description

Listeria monocytogenes is a Gram-positive, non-spore forming bacterium that is widespread in the natural environment and carried by many domestic and wild animals. It has been isolated from soils and vegetation, animal feeds such as silage, as well as surface and marine waters as a result of contamination from sewerage or run off.

L. monocytogenes grows at low oxygen conditions and refrigeration temperatures (<5 °C). It can survive for long periods in the environment, on foods, and in food processing plants where it has been isolated from floors, drains, wet processing areas and equipment. Post-processing contamination at food contact surfaces is a main factor for the presence of *L. monocytogenes* in ready-to-eat foods.

L. monocytogenes causes listeriosis, which may be non-invasive (a mild form of disease) or invasive. Invasive listeriosis is a relatively rare but often severe disease with fatality rates around 20-30%. Populations at risk include those with chronic disease (e.g. cancer, diabetes, malnutrition, AIDS); pregnant women (foetuses or neonates infected *in utero*); the elderly and individuals being treated with immunosuppressive drugs (e.g. transplant patients).

Individuals infected with *L. monocytogenes* may exhibit mild flu-like symptoms such as fever and muscle aches, and sometimes gastrointestinal symptoms such as vomiting and diarrhoea. In at-risk population groups manifestations of the more severe, invasive form of the disease include bacteraemia, septicaemia, meningitis, encephalitis, miscarriage, neonatal disease, premature birth, and stillbirth.

Illness is generally associated with the ingesting high numbers of *L. monocytogenes*. For invasive listeriosis, the level will vary depending on the virulence of the serotype and the general health and immune status of the host.

Associated foods

Outbreaks of foodborne listeriosis have included those associated with soft style cheeses, delicatessen meats, cooked chicken, pre-prepared salads, pâté, smoked seafood and rockmelon.

Risk factors typically associated with foods linked to outbreaks include:

- it is ready-to-eat
- it has an extended shelf life at refrigeration temperatures
- it is susceptible to post-process contamination or has received no listericidal processing
- Product characteristics support the growth of *L. monocytogenes* to levels that can present a risk to consumers.

Control measures

Control of *L. monocytogenes* in ready-to-eat foods includes:

- minimising contamination of raw materials during primary production
- using listericidal processes
- minimising contamination following processing
- restricting growth through limiting shelf life, maintaining the cold chain or product formulation.

For ready-to-eat foods that have received a listericidal process (such as cooking or pasteurisation), control measures should minimise post-process contamination before final packaging or during subsequent handling. These should include the design and maintenance of premises and equipment, process flow, and cleaning and sanitation programs. The *Codex Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* outlines key control measures to minimise or prevent contamination and growth of *L. monocytogenes* in ready-to-eat foods.

Foods with the following criteria are regarded as not being able to support the growth of *L. monocytogenes*:

- pH < 4.4
- aw < 0.92
- a combination of pH < 5.0 and water activity < 0.94.

Resources

[Codex \(2007\) Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods \(CAC/GL 61 – 2007\) Codex Alimentarius Commission, Geneva, Switzerland.](http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/)

<http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/>

ICMSF (1996) *Microorganisms in Foods 5: Microbiological specifications of food pathogens*. Chapman & Hall, London.

[FDA \(2012\) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 83-86](http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2013\) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.](http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

[New Zealand Ministry of Primary Industries \(2001\) Listeria monocytogenes Microbial Pathogen Data Sheet.](http://www.foodsafety.govt.nz/elibrary/industry/Listeria_Monocytogenes-Science_Research.pdf)

http://www.foodsafety.govt.nz/elibrary/industry/Listeria_Monocytogenes-Science_Research.pdf

Sutherland P, Miles D and Laboyrie (2003). *Listeria monocytogenes*. Ch 13 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney,

Salmonella spp. (non-typhoidal)

Description

Salmonella spp. are members of the family *Enterobacteriaceae*. They are Gram negative non-spore forming rod-shaped bacteria, generally motile.

Salmonella spp. are named and typed according to antigenic typing and subtyped further through phage typing or molecular typing, such as pulse-field gel electrophoresis (PFGE). More recently, whole genome sequencing (WGS) is being used as a sub-typing tool. Over 2500 serotypes of salmonella have been described.

Salmonella Typhi, the agent causing typhoid fever, is the only *Salmonella* serovar for which humans are the only animal carrier. *Salmonella* spp. associated with gastrointestinal foodborne illness are termed non-typhoidal *Salmonella* (and are members of the species *Salmonella enterica*) In Australia, *S. Typhimurium* is the most commonly reported serovar of all notified *Salmonella* infections.

A primary reservoir for *Salmonella* is the intestinal tract of vertebrates, including poultry, livestock, wildlife, domestic pets, and humans. Faecal shedding by animals colonised with *Salmonella* spp. leads to contamination of the surrounding environment including soil, crops and water ways. *Salmonella* can survive for long periods of time in foods and other substrates.

Salmonella spp. are transmitted via consumption of contaminated food or water, as well as person-to-person contact or from direct contact with infected animals. Gastrointestinal illness results when *Salmonella* are able to invade the intestinal epithelial cells and infect the host, producing a heat labile enterotoxin. Low numbers of *Salmonella* may cause illness.

Symptoms of salmonellosis include nausea, vomiting, diarrhoea, cramps and fever. The duration of these symptoms is several days.

Associated foods

A wide range of foods have been implicated in foodborne salmonellosis, particularly those of animal origin and foods that have been subject to faecal contamination from the environment. Examples of foods that have been attributed to outbreaks of illness include:

- animal products such as eggs (particularly raw egg dishes), poultry, raw meat, milk and dairy products
- fresh produce (such as leafy greens, seed sprouts, melons, paw paw)
- low moisture foods such as spices, peanut butter, chocolate.

Factors contributing to salmonellosis include:

- cross contamination during food handling (from the environment or raw products)
- inadequate temperature control
- inadequate processing
- consumption of contaminated raw products.

Control Measures

Control of *Salmonella* includes:

- prevention of contamination (particularly of ready-to-eat foods)
- including a processing step that will kill any *Salmonella* that may be present
- maintaining temperature control to prevent growth.

Cross contamination occurs when *Salmonella* is spread to foods via contaminated food (such as raw meat, poultry or eggs), water, animals or an infected food handler. Contamination can spread further to food contact surfaces, equipment and utensils if there is inadequate cleaning and sanitising or inadequate hygiene practices such as hand washing.

Growth and survival characteristics

- temperature

The optimal growth temperature for *Salmonella* spp. is 35 to 43°C. Most serotypes do not grow at temperatures below 7°C. Growth of *Salmonella* spp. does not occur at 50°C. Foods that are high in fat and low in moisture, such as chocolate and peanut butter, may have a protective effect against heat.

- pH

Salmonella spp. will grow over a broad pH range however the optimum pH for growth is 7 – 7.5. The minimum pH at which *Salmonella* spp. can grow is dependent on temperature, presence of salt and nitrite and the type of acid present and has been reported as pH 3.8.

- water activity

The optimum water activity for growth of *Salmonella* spp. is 0.99. The minimum water activity for growth is 0.93. *Salmonella* spp. can survive for extended periods in foods with a low water activity, such as black pepper, chocolate, peanut butter and gelatine.

Resources

ICMSF (1996) *Microorganisms in Foods 5: Microbiological specifications of food pathogens*. Chapman & Hall, London.

[FDA \(2012\) *Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 83-86*](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2013\) *Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.*](#)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

Jay L,S, Davos D, Dundas M, Frankish E, Lightfoot, D (2003). *Salmonella*. Ch 8 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p207 - 266

[New Zealand Ministry of Primary Industries \(2001\) *Non Typhoidal Salmonellae Microbial Pathogen Data Sheet.*](#)

<http://www.foodsafety.govt.nz/elibrary/industry/non-typhoid-salmonellae.pdf>

Shiga Toxin-producing *Escherichia coli* (STEC)

Description

Escherichia coli are rod-shaped, Gram-negative bacteria that occur naturally in the gut of humans and warm-blooded animals. Some *E. coli* strains are pathogenic because they have acquired virulence factors and are grouped based on how they cause disease and the symptoms that occur. These pathogenic strains are further serotyped based on three antigens: O (somatic), H (flagella) and K (capsule) antigens. Usually the O and H antigens are enough to classify the strain.

Shiga toxin-producing *E. coli* (STEC) also known as verocytotoxin-producing *E. coli* (VTEC) are strains of *E. coli* that produce Shiga toxins (Stx). These pathogenic *E. coli* are able to cause serious disease in humans including haemorrhagic colitis (HC). The highest amount of STEC infections globally are caused by STEC O157 strains (in particular O157:H7). Other strains associated with illness in Australia include O111, O26, O113, O55 and O86. The term EHEC is commonly used to refer to the subgroup of STEC that cause HC and includes the serotypes O157:H7, O26:H11, O111:H-, O157:H-.

Symptoms of STEC infection include abdominal cramps, (bloody) diarrhoea, vomiting and fever. The illness develops over 3–8 days, with many patients improving in 10 days. However more serious illness may result, including haemolytic uraemic syndrome (HUS) and its associated complications. In some individuals this can lead to kidney failure and death. Children under five years of age and the elderly are more susceptible to infection and the development of serious illness.

The dose required for STEC to cause illness will depend on the serotype and virulence factors. For *E. coli* O157:H7 the infective dose is estimated to be low (10 – 100 cells).

The major animal reservoir of STEC is ruminants, in particular cattle and sheep. Infected animals shed the bacteria in their faeces, resulting in contamination of the environment. STEC can survive in soil, manure, water trough sediments and can also survive for extended time in water.

Direct transmission of STEC to humans is possible through contact with infected animals as well as person to person. The major transmission route is foodborne.

Associated foods

Foods that have been associated with STEC outbreaks include those of animal origin and fresh produce that has been subject to faecal contamination from the environment:

- inadequately cooked ground beef (hamburger patties)
- poorly processed uncooked fermented comminuted meat (e.g. salami)
- raw or inadequately pasteurised dairy products
- fresh produce such as leafy greens and sprouted seeds.

Control Measures

The main source for STEC and entry point into the food chain is animal faeces. Primary produce can be either contaminated directly by faecal material or indirectly via contaminated water or soil.

Control measures for STEC are through chain and include:

- preventing/minimising contamination of raw products at primary production by implementing good hygienic practices
- ensuring processing controls are adequate (e.g. cooking, pasteurisation, fermentation [including control of pH, water activity etc.]).
- preventing cross contamination of RTE foods from raw foods and the processing environment.

Growth and survival characteristics

- temperature

E. coli does not grow at temperatures below 7 °C but can survive in chilled and frozen food. Optimum temperatures for growth are 35–40 °C. Its sensitivity to heat depends on the composition, pH and water activity of the food, for example the heat resistance increases as the a_w decreases. It is generally recommended that foods (such as hamburger patties) are thoroughly cooked to a core temperature of 75°C.

- pH and water activity

The optimum pH range for *E. coli* growth is pH 6–7 with the minimum pH for growth being 4.4. The effect of pH on survival however depends on the acid present, for example STEC are more acid resistant when hydrochloric acid is the acidulant than when lactic acid is used. The minimum water activity permitting growth of *E. coli* is 0.95 (about 8% salt). This value increases as pH and temperature conditions become sub-optimal.

Resources

[FDA \(2012\) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79.](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2013\) Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.](#)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

Staphylococcus aureus

Description

Staphylococcus aureus is a Gram-positive, non-spore forming cocci bacteria that belongs to the *Staphylococcus* genus. Several staphylococcal species (coagulase-negative and coagulase-positive strains) have the ability to produce heat-stable enterotoxins that cause gastroenteritis in humans. Staphylococcal food poisoning is predominantly caused by *S. aureus*.

Staphylococci are widespread in the environment and commonly occur on the skin and mucous membranes of warm-blooded animals. Humans are a main source of enterotoxin producing strains, with many healthy people (50% or more) carrying *S. aureus* as part of the normal microflora of the nose, throat or skin. *S. aureus* can survive for extended periods in a dry state.

Staphylococcal enterotoxins (SEs) are produced in food during the exponential phase of *S. aureus* growth. Doses of SE that cause illness are reached when *S. aureus* grows to levels of $10^5 - 10^8$ cfu/g. SEs are very resistant to freezing and heating and will survive thermal processes used for low acid canned foods.

Staphylococcal food poisoning occurs following ingestion of food containing SEs. There is generally a rapid onset of symptoms, appearing around 3 hours after ingestion (range 1–7 hours) which include nausea, vomiting, abdominal cramps and diarrhoea. While illness is acute, it is generally self-limiting and recovery is rapid (within 2 days).

Associated foods

All foods that are handled directly by humans and/or those of animal origin may be contaminated with staphylococci.

Foods associated with Staphylococcal food poisoning are those that often require considerable handling during preparation and are prone to be out of refrigeration for extended periods. Such foods may include bakery products such as cream or custard filled pies and éclairs; sandwich fillings; meat, poultry and egg products; salads such as potato, tuna, chicken and pasta. Foods high in starch and protein are thought to favour SE production.

Control Measures

Temperature

S. aureus grows in the temperature range 7°C – 48°C, with optimal growth between 35°C - 40°C. The production of enterotoxin is optimal between 40–45°C and does not occur at temperatures <10°C. As temperature decreases, the level of SE production also decreases. *S. aureus* is easily killed at pasteurisation or cooking temperatures.

The time food products prone to contamination by *S. aureus* are held at temperatures between 5°C and 60°C should be minimised in order to prevent the opportunity for *S. aureus* growth and toxin production. The '4-hour/2-hour rule' (Appendix 1 of *Safe Food Australia*) provides time limits that can be applied for when RTE food is outside of temperature control.

Heat processes such as cooking and pasteurisation will destroy viable cells of *S. aureus* but will not destroy preformed staphylococcal enterotoxins.

Hygiene of food handlers

Food handlers are regarded as the main source of food contamination with *S. aureus*. Food handling controls to minimise contamination during food preparation include:

- preventing unnecessary contact with RTE food
- using gloves, tongs or other implements to handle food
- handwashing whenever direct contact with food is likely to occur
- avoiding sneezing, coughing or blowing over food or food contact surfaces.

Other growth characteristics

S. aureus is tolerant of high salt and sugar content and can grow in conditions of low water activity. Most *S. aureus* strains can grow over a a_w range of 0.83 (when other conditions are optimal) to >0.99.

Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of pH 6–7.

Resources

ICMSF (1996) *Microorganisms in Foods 5: Microbiological specifications of food pathogens*. Chapman & Hall, London.

[FDA \(2012\) *Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79.*](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

[FSANZ \(2001\) *Safe Food Australia. 2nd ed, Food Standards Australia New Zealand Canberra.*](#)

<http://www.foodstandards.gov.au/publications/Pages/safefoodaustralia2nd519.aspx>

[FSANZ \(2013\) *Agents of foodborne illness. 2nd ed, Food Standards Australia New Zealand, Canberra.*](#)

<http://www.foodstandards.gov.au/publications/pages/agentsoffoodborneill5155.aspx>

[New Zealand Ministry of Primary Industries \(2001\) *Staphylococcus aureus Microbial Pathogen Data Sheet.*](#)

http://www.foodsafety.govt.nz/elibrary/industry/Staphylococcus_Aureus-Science_Research.htm

Stewart, C (2003) *Staphylococcus aureus and Staphylococcal Enterotoxins*. Ch 12 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p359 - 380

Vibrio parahaemolyticus

Description

Vibrio parahaemolyticus is a Gram-negative, curve-shaped rod naturally present in coastal and estuarine waters. *V. parahaemolyticus* is halophilic (salt-tolerant) and is lysed almost immediately in freshwater. It is a natural contaminant of seafood (fish, shellfish and crustaceans).

Most *V. parahaemolyticus* isolates from the environment are non-pathogenic. Pathogenic strains (indicated by the Kanagawa reaction or presence of genetic markers) cause gastroenteric infections, with symptoms including diarrhoea (which can be bloody), abdominal pain, nausea and vomiting. The infectious dose is greater than 10^5 viable pathogenic cells with onset of symptoms ranging from 4 hours to a couple of days.

The initial levels of *V. parahaemolyticus* in seafood will depend on environmental factors at harvest, such as water temperature and salinity, and vary seasonally. Numbers naturally present in seafood are generally low (<100 cfu/g).

V. parahaemolyticus grows at temperatures between 5 – 43°C, with optimal growth at 37°C. *V. parahaemolyticus* can grow rapidly and under optimum condition has a generation time of 9 to 10 minutes. This means it can increase to infective levels within a short period (2 to 3 hours) in warm conditions. It is slowly inactivated at temperatures below 7°C.

Associated foods

Foods associated with foodborne illness caused by *V. parahaemolyticus* are predominantly fish, shellfish and crustaceans (particularly raw molluscs and cooked crustacea).

Control Measures

One of the main control measures for *V. parahaemolyticus* is to chill seafoods quickly to <5°C after harvest and maintain them under refrigeration to prevent growth. Shellfish harvesting practices may also be implemented to ensure that shellfish is not harvested where water temperatures are in a particular range or following a rainfall event in estuarine areas.

Cooking to an internal temperature of 65°C will destroy any *V. parahaemolyticus* present.

Cross contamination should be prevented by keeping raw and cooked foods separate and preventing transfer from food contact surfaces.

Resources

Desmarchelier P (2003) *Pathogenic vibrios*. Ch 11 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p333 - 358

[FDA \(2012\) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed, US Food and Drug Administration, Silver Spring, p. 75–79.](#)

<http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm>

ICMSF (1996) Microorganisms in Foods 5: Microbiological specifications of food pathogens. Chapman & Hall, London.

[New Zealand Ministry of Primary Industries \(2001\) *Vibrio parahaemolyticus* Microbial Pathogen Data Sheet](#)

http://www.foodsafety.govt.nz/elibrary/industry/Vibrio_Parahaemolyticus-Science_Research.htm

APPENDIX 2 Indicator microorganisms

Direct testing of pathogens is not always possible or practical. The use of indicator and index tests can be a useful and cost effective means of assessing the microbiological status of food. They can be used to:

- indicate the effectiveness or otherwise of process hygiene and process controls (indicator microorganisms)
- indicate the presence of pathogenic microorganisms when direct and reliable analytical methods are not available (index microorganisms).

Information on indicator microorganisms commonly tested in foods is provided below.

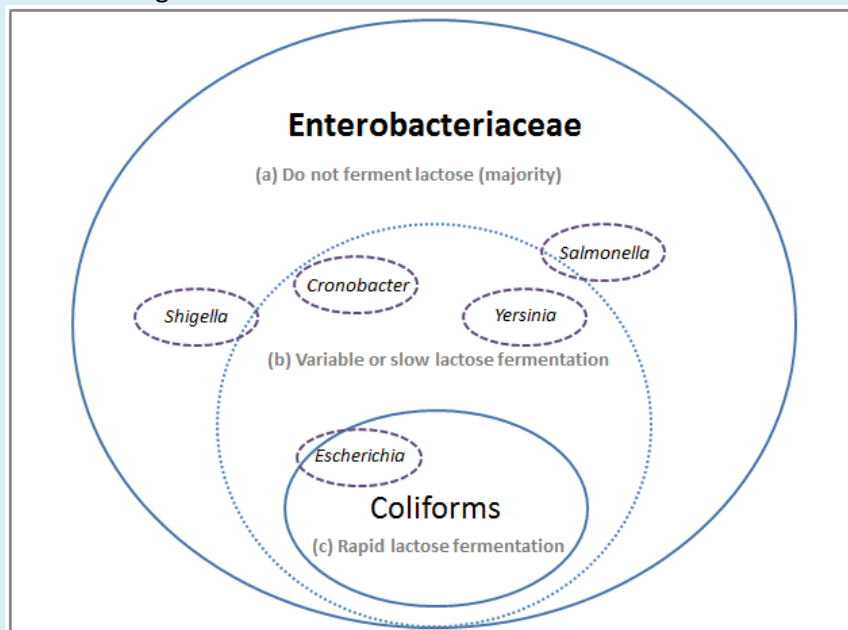
Enterobacteriaceae

Description

Enterobacteriaceae is a family of gram-negative, non-spore forming bacteria that includes many bacteria that are found in human or animal intestinal tracts, as well as plants and the environment. The family includes a number of foodborne pathogens such as *Salmonella*, pathogenic *E. coli*, *Shigella* and *Cronobacter*, as well as non-pathogenic bacteria.

The ability of Enterobacteriaceae to produce acid and gas from the fermentation of D-glucose is a characteristic commonly used as a basis for their detection and enumeration. Enterobacteriaceae also lack cytochrome C oxidase (have a negative reaction to the oxidase test) which enables them to be differentiated from other closely related bacteria.

While most Enterobacteriaceae do not ferment lactose, some members (collectively termed coliforms) are able to ferment lactose rapidly (within 24 – 48 hours) producing acid and gas. Members of the Enterobacteriaceae that do not ferment lactose, or ferment it slowly include pathogens (e.g. *Salmonella*, *Shigella*, and some pathogenic *E. coli*) which aren't detected by coliform tests. The relationship between members within the Enterobacteriaceae and those in the coliform group is depicted in the diagram below.



Source: Adapted from Baylis *et al* (2011)

Purpose of test

Enterobacteriaceae counts are useful to assess the adequacy of processing and hygiene practices, particularly for heat treated foods. As all Enterobacteriaceae are killed by thermal processes used in food production, their presence in pasteurised or cooked foods can indicate inadequate processing or post-process contamination.

Interpretation of results

The significance of testing results for Enterobacteriaceae will depend on the type of food being analysed. For example high levels of these bacteria are expected in some food commodities such as salad vegetables and other foods of plant origin.

There are also psychrotrophic Enterobacteriaceae that are able to multiply in chilled foods. These are widely distributed and found in a variety of foods including milk, meat and poultry. This makes it difficult to interpret levels found throughout the shelf life of a chilled food as they do not necessarily reflect initial contamination levels or whether temperature control has been adequate.

Enterobacteriaceae do provide an indication of processing and good hygiene on the day of production.

Resources

[Baylis C, Uyttendaele M, Joosten H, & Davies A \(2011\) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute \(ILSI\), Europe.](http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/)

<http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/>

Craven H, Eyles M, & Davey J (2003) *Enteric Indicator Organisms in Foods*. Ch 6 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 163-194

Coliforms

Description

Coliforms are a group of Enterobacteriaceae that are able to ferment lactose rapidly (within 24 – 48 hours) producing acid and gas. They are not a well-defined taxonomic group and are often defined by the method used (e.g. ability to ferment lactose rapidly). Bacteria outside the Enterobacteriaceae group can also ferment lactose and can be falsely detected as coliforms if no other confirmatory tests are performed.

Organisms that ferment lactose (presumptive coliforms) may be inoculated into selective media at temperatures between 44 – 45.4 °C. If lactose fermentation occurs at these elevated temperatures, the organisms are termed faecal or thermophilic coliforms. Faecal coliforms may be tested further to determine whether they are *E. coli*.

Purpose of test

Historically, coliforms were the most common indicator group tested for by the food industry, particularly by the dairy sector for monitoring the effectiveness of hygiene measures post pasteurisation.

A high coliform count in heat processed foods generally indicates under processing or unsatisfactory post-process contamination.

Interpretation of results

The presence of coliforms in many foods may be expected and does not necessarily indicate unsatisfactory hygiene measures. For example coliforms are part of the normal flora of many raw foods including cereal crops and vegetables and are generally present on raw meats as well as some fermented foods.

Coliforms are able to survive and grow in food processing environments where other pathogenic Enterobacteriaceae may not. As such, their presence in food does not necessarily indicate faecal contamination. Their presence at high levels provides a warning that unhygienic food handling may have occurred or processing was not effective.

Resources

[Baylis C, Uyttendaele M, Joosten H, & Davies A \(2011\) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute \(ILSI\), Europe.](http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/)

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Craven H, Eyles M, & Davey J (2003) *Enteric Indicator Organisms in Foods*. Ch 6 In: Hocking AD (ed) *Foodborne microorganisms of public health significance*. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, p. 163-194

Escherichia coli

Description

E. coli are gram-negative, facultative anaerobic rod shaped bacteria that are a common part of the normal intestinal flora of humans and other warm-blooded animals. As such, *E. coli* is a more specific indicator of faecal contamination than Enterobacteriaceae or coliforms. Its presence in foods indicates recent contamination, either directly or indirectly by faeces or faecally contaminated material.

E. coli can become established in processing environments and can grow on inadequately cleaned surfaces and in food. It is killed by thermal processes used in food production and can be readily removed from food processing equipment and surfaces by appropriate cleaning procedures.

Purpose of test

E. coli testing is used predominantly as an indicator of faecal contamination and measure of the effectiveness of hygiene measures. This can be useful for raw commodities as well as heat processed foods to indicate:

- GMP/GHP of meat slaughter practices
- potential faecal contamination of raw fruit and vegetables during growth and harvest (GAP)
- potential faecal contamination of bivalve mollusc harvest waters
- post-process contamination or inadequate processing of heat-treated foods.

E. coli has also been used as an index organism for enteric pathogens such as *Salmonella*.

Interpretation of results

E. coli is the best indicator of recent faecal contamination. It can, however, become established in factory environments such that its presence does not necessarily signify faecal contamination or the risk of enteric pathogens being present. Equally, the absence of *E. coli* does not ensure that enteric pathogens are not present as the survival and growth characteristics of different strains of *E. coli* and enteric pathogens can vary.

Interpretation of *E. coli* results should relate to the purpose of the test and the risk implied by the presence or level detected. For some foods or processes (e.g. production of uncooked comminuted fermented meat) the detection or level detected may require further testing of specific pathogens such as STEC or Salmonella.

Resources

[Baylis C, Uyttendaele M, Joosten H, & Davies A \(2011\) *The Enterobacteriaceae and their significance to the food industry*. International Life Sciences Institute \(ILSI\), Europe.](http://ilsi.org/publication/the-enterobacteriaceae-and-their-significance-to-the-food-industry/)

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Mesophilic Aerobic Bacteria

Description

The mesophilic aerobic bacteria (MAB) count, also referred to as aerobic plate count, total viable count or standard plate count is used to indicate the level of bacteria in a food product. It provides the number of bacteria that grow in the presence of oxygen (aerobic) and at moderate temperatures (mesophilic). Plates are generally incubated at 30°C. The test is based on bacterial cells present forming visible colonies when mixed with agar containing appropriate nutrients. Different types of bacteria are not differentiated.

Purpose of test

MAB is one of the most common tests applied to indicate microbiological quality of food. It does not determine the presence of pathogenic microorganisms and should not be used as a direct assessment of safety.

The significance of MAB counts varies markedly according to the type of food product and the processing it has received. If it is applied on a regular basis it can be a useful means of observing trends by comparing results over time.

Interpretation of results

Interpretation of MAB counts should take into consideration knowledge of the product and whether a high count is expected. The stage of shelf life should also be considered as the MAB will increase over the life of a food product (unless processing factors prevent growth e.g. dried food products).

For raw commodities, such as fruits, vegetables, raw meat and fish, total counts are likely to be quite high due to the bacterial flora normally present (10^6 - 10^7 cfu/g). Fermented foods will also have high colony counts, mainly comprising the starter culture used.

Foods that have received heat treatments such as pasteurisation or cooking should have low MAB counts following processing ($<10^4$ cfu/g).

MAB counts in foods that undergo considerable handling such as slicing, portioning, packaging etc. will be influenced by the hygiene measures in place. Table 3 *Interpretation of results for mesophilic aerobic bacteria count in RTE foods* in Section 3 below provides further information.

Resources

[Health Protection Agency \(2009\) Guidelines for Assessing the Microbiological Safety of Ready-to-eat Foods Placed on the Market. Health Protection Agency, London.](https://www.gov.uk/government/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines)

<https://www.gov.uk/government/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines>