

# Microbiological Guidelines for Food

(For ready-to-eat food in general and specific food items)

XXX 2014

(revised)

Centre for Food Safety

*This set of Guidelines is published by  
the Centre for Food Safety, Food and Environmental Hygiene Department.  
The technical content has been developed  
in consultation with  
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# **Microbiological Guidelines for Food**

## Preface

As part of the Government's ongoing efforts to enhance food safety for the protection of public health and consumer interest, the Administration conducts regular review on microbiological standards and guidelines for food and amends them where necessary by taking international/ national standards and guidelines as well as local situation into consideration.

In light of changing needs and expert views, the Microbiological Guidelines for Ready-to-eat Food (the Guidelines) established in 2002 were amended under the advice of the Expert Panel on Microbiological Safety of Food in 2007. Subsequently in 2009, supplementary information to the Guidelines regarding the microbiological criteria for bottled waters and edible ice and the revised microbiological criteria for non-bottled drinks were established under the advice of an Ad Hoc Working Group on Microbiological Safety of Food formed under the Expert Committee on Food Safety (Expert Committee) in 2008.

In order to keep the local microbiological guidelines abreast of the international development and advancement of food science and technology, the Ad Hoc Working Group on Microbiological Safety of Food 2011 (Working Group) was formed under the Expert Committee to provide professional recommendation based on the latest situation.

Apart from textual amendments, major revisions on the following aspects were recommended by the Working Group - (1) modifying the existing microbiological criteria as well as establishing additional microbiological criteria with reference to international/ national standards and guidelines, (2) incorporating microbiological criteria stipulated in the supplementary information to the Guidelines, (3) revising the classification and nomenclature of microbiological quality and (4) putting in additional information on common foodborne pathogens in Appendix I for reference. The trade was consulted on the proposed amendments and the Guidelines were endorsed by the Expert Committee. Since the revised Guidelines would include microbiological criteria for both ready-to-eat and non-ready-to-eat food e.g. powdered infant formula, the title of the Guidelines is also changed to "Microbiological Guidelines for Food".

The revised Guidelines supersede those previously issued and serve to facilitate enforcement in monitoring and controlling of microbiological quality of food as well as facilitating the trade in devising measures to improve food safety.

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## **Ad Hoc Working Group on Microbiological Safety of Food 2011**

The Expert Committee on Food Safety (Expert Committee), set up under the Centre for Food Safety, is responsible for advising the Director of Food and Environmental Hygiene on the formulation of food safety measures, review of food safety standards in light of international practices, trends and developments, as well as risk communication strategies. In 2011, the Expert Committee endorsed setting up the Ad Hoc Working Group on Microbiological Safety of Food 2011 (Working Group) to provide advice on the review of “Microbiological Guidelines for Ready-to-eat Food” (the Guidelines) in Hong Kong.

The Working Group consists of academics, professionals, representatives from Government Departments and members of the Expert Committee. The Working Group is chaired by Consultant (Community Medicine) (Risk Assessment and Communication) with secretariat support provided by Risk Assessment Section of the Centre for Food Safety. Officials from the Department of Health and Agriculture, Fisheries and Conservation Department provide advice on issues that fall under their purviews.

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## **Introduction to Microbiological Guidelines for Food**

### **- Recommendations for Food Safety Monitoring in Hong Kong-**

Food safety control aims to safeguard public health and provide assurance on food safety. To this end, microbiological analyses are useful ways to assess the safety and quality of food involved. This set of Guidelines presents the recommended microbiological criteria for (1) ready-to-eat food in general and (2) specific food items.

#### **Purpose of the Guidelines**

2. In the Hong Kong Special Administrative Region, the legal powers and instruments for the enforcement of microbiological safety of food are provided for in the Public Health and Municipal Services Ordinance, Chapter 132 (the Ordinance). Section 54 of the Ordinance stipulates that it is an offence to sell food that is unfit for human consumption. General protection for purchasers of food is provided in Section 52 of the Ordinance when a person may be guilty for selling to the prejudice of a purchaser any food which is not of the nature, substance or quality demanded by the purchaser. Legal microbiological standards for some specified foods are stipulated in its subsidiary legislation.

3. Microbiological Guidelines are criteria indicating the microbiological condition of the food items when there are no established microbiological standards. They also supplement any existing legislative microbiological standards so as to reflect the safety and hygienic quality of the food. The purpose of this set of Guidelines is to provide assistance to officers in the interpretation of microbiological analyses of foods and give recommendations on the appropriate follow-up action to monitor and control food safety. It also serves to facilitate the trade in devising measures to improve their food safety practices.

#### **Use of the Guidelines**

4. The microbiological criteria for (i) ready-to-eat food in general - Aerobic colony count (ACC) and Hygiene indicator organisms, (ii) ready-to-eat food in general – specific foodborne pathogens and (iii) specific food items are

listed in Chapters I to III respectively.

5. Microbiological methods are appended to some microbiological criteria which are given in the relevant Codex standards and code of hygienic practices. Other equivalent methods that have been validated to provide appropriate sensitivity, reproducibility, reliability etc. could be employed. Preference should be given to methods which have been validated for the commodity concerned especially in relation to reference methods elaborated by international organisations. This is also applicable to other microbiological criteria in this set of Guidelines, which have no microbiological method appended.

6. Additional information on the Common Foodborne Pathogens (Appendix I) and the Guidance Notes on Sampling Plan for Microbiological Analysis (Appendix II) are supplemented to this set of Guidelines for reference.

## **Chapter I. Microbiological Criteria for Ready-to-eat Food in General - Aerobic Colony Count (ACC) and Hygiene Indicator Organisms**

### **Introduction**

Microbiological criteria in Chapters I and II are intended for assessing the microbiological quality of ready-to-eat food in general. These criteria were based on the local “Microbiological Guidelines for Ready-to-eat Food (May 2007 Revised)” and revised with reference to the “Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market”<sup>1</sup> published by the Health Protection Agency in the United Kingdom in November 2009 and the advice from the Ad Hoc Working Group on Microbiological Safety of Food 2011 after taking local situation into consideration.

2. The criteria stated in Chapters I and II apply to ready-to-eat food in general. “Ready-to-eat food” means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level the microorganisms of concern.<sup>1</sup>

3. However, some microbiological criteria set out in Chapter III apply to certain food items which may not be ready-to-eat. Chapters I, II and III should be read together for the microbiological criteria of specified food. For example, for live or raw bivalve molluscs intended for direct consumption, the relevant *Escherichia coli* and *Salmonella* spp. criteria are stipulated in Chapter III. As regards other microbiological criteria, they are set out in Chapters I and II.

### **Components of Microbiological Criteria for Ready-to-eat Food in General**

4. The microbiological limits for ready-to-eat food in general consist of three components:

- ◆ Aerobic colony count (ACC);
- ◆ Hygiene indicator organisms – *E. coli* and Enterobacteriaceae;
- ◆ Specific foodborne pathogens – ten specific bacterial pathogens.

5. The microbiological criteria for ACC and hygiene indicator organisms are covered in this Chapter while those for specific foodborne pathogens are included in Chapter II.

### **Classification of Microbiological Quality**

6. The microbiological assessment of ready-to-eat food on the above three components will lead to the classification of microbiological quality into one of the following three classes<sup>1</sup>:

- (a) **Satisfactory:** test results indicating good microbiological quality.
- (b) **Borderline:** test results that are not unsatisfactory but also not fully satisfactory. Test results that are on the upper limit of acceptability and which indicate potential for development of public health problems and unacceptable risk.
- (c) **Unsatisfactory:** For ACC, test results which call for action to consider investigating the reasons for the high count. For hygiene indicators, test results that require remedial action. For pathogens, test results that are at levels indicating that a product is potentially injurious to health and/or unfit for human consumption and require immediate remedial action.

7. Suggested actions to be taken for each class are summarised in Table 1.1.

**Table 1.1 Summary on suggested actions (not exclusive) to be taken for each class in response to ACC, hygiene indicator organisms and specific foodborne pathogens results in ready-to-eat foods**

	ACC	Hygiene indicator organisms	Specific foodborne pathogens (NB: Perform risk assessment before any further action)
<b>Satisfactory</b>	No action required.		
<b>Borderline</b>	Consider the source of the food (producer/ retailer etc.) and the stage of shelf life before determining action. If other samples from the same source are also of borderline quality, further investigation may be appropriate.	Parties concerned (e.g. vendors) should be informed to review cooking and all hygiene procedures including cleaning. Consider taking investigative food samples. Action should be proportional to the levels detected.	Risk will increase proportional to the levels detected. Parties concerned (e.g. vendors) should be warned to investigate and find out the causes and adopt measures to improve the situation. Consider taking investigative food samples.
<b>Unsatisfactory</b>	Consider investigating the reasons for the high count.	Parties concerned (e.g. vendors) should be warned to review cooking and all hygiene procedures including cleaning. Take investigative food samples.	Immediate investigation; Parties concerned (e.g. vendors) should be instructed to stop sale of food item in question, investigate immediately and find out the causes and adopt measures to improve the situation. Take investigative food samples. In addition, warning letters, source tracing and other enforcement actions should be considered.

## **Aerobic Colony Count (ACC)**

“Aerobic colony count (ACC)”, also known as the total viable count or standard plate count, is the total number of bacteria able to grow in an aerobic environment in moderate temperature. It is an indicator of quality, not safety, and cannot directly contribute towards a safety assessment of ready-to-eat food. In addition, ACCs can provide useful information about the general quality and remaining shelf life of the food in question, and thus highlight potential problems of storage and handling since production; however they are not deemed a priority in a risk based analysis.<sup>1</sup>

2. In general, immediate action in response to high ACCs is not usually warranted except for shelf-stable canned or bottled food products immediately after opening (Food Category 1, Table 1.2). The ACC level in ready-to-eat foods will depend initially on the type and duration of processing that the food has received during production. Thereafter the level will depend on how it is handled and stored.<sup>1</sup>

3. Guidance on the interpretation of results for ACC levels in various ready-to-eat foods is provided in Table 1.2.

**Table 1.2 Guidance on the interpretation of results for ACC levels [30°C/48 hours] in various ready-to-eat foods**

Food Category <sup>a</sup>	Examples	Result (colony-forming unit (cfu)/g)		
		Satisfactory	Borderline	Unsatisfactory
1. Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container <sup>b</sup>	Canned products such as tuna, salmon, corned beef, soups, stews, desserts and fruit; ultra-high-temperature (UHT) products	<10	N/A	Note <sup>c</sup>
2. Foods cooked immediately prior to sale or consumption	Takeaway food, burgers, kebabs, sausages, pizza, ready meals (cook/chill and cook/freeze) after regeneration, dim sum, rice, noodles	<10 <sup>3</sup>	10 <sup>3</sup> -<10 <sup>5</sup>	≥10 <sup>5</sup>
3. Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham requiring refrigeration, pasteurised foods including fruit juice and soups; desserts	<10 <sup>4</sup>	10 <sup>4</sup> -<10 <sup>7</sup>	≥10 <sup>7</sup>
4. Bakery and confectionery products without dairy cream, powdered foods	Cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready to eat after reconstitution or warming	<10 <sup>4</sup>	10 <sup>4</sup> -<10 <sup>6</sup>	≥10 <sup>6</sup>
5. Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (mackerel, etc.), molluscs, crustaceans and other shellfish out of shell, non-prepackaged cold beverages with solid ingredients but without dairy components (iced green tea with red bean, etc.)	<10 <sup>5</sup>	10 <sup>5</sup> -<10 <sup>7</sup>	≥10 <sup>7</sup>
6. Non-fermented dairy products and dairy desserts, mayonnaise and mayonnaise based dressings, cooked sauces	Most butter, fresh cheese (mascarpone, paneer), trifle with dairy cream, satay, cakes with dairy cream, non-prepackaged cold beverages with solid ingredients and dairy components (iced milk tea with pearl tapioca, etc.)	<10 <sup>5</sup>	10 <sup>5</sup> -<10 <sup>7</sup>	≥10 <sup>7</sup>
7. Food mixed with dressings, dips, pastes	Coleslaw, dips, taramasalata, houmous	<10 <sup>6</sup>	10 <sup>6</sup> -<10 <sup>7</sup>	≥10 <sup>7</sup>
8. Extended shelf life food products requiring refrigeration	Modified atmosphere packaging (MAP) or vacuum packed products, e.g. meat, fish, fruit and vegetables	<10 <sup>6</sup>	10 <sup>6</sup> -<10 <sup>8</sup>	≥10 <sup>8 d</sup>
9. Raw ready-to-eat meat and fish, cold smoked fish	Sushi, sashimi, smoked salmon, gravalax	<10 <sup>6</sup>	10 <sup>6</sup> -<10 <sup>7</sup>	≥10 <sup>7</sup>
10. Preserved food products – pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices	N/A	N/A	N/A
11. Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish	N/A	N/A	N/A
12. Fresh fruit and vegetables, products containing raw vegetables	Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables, non-prepackaged cold beverages with solid and fresh fruit ingredients	N/A	N/A	N/A

	(chilled fresh mango juice with pomelo and sago, etc.)			
13. Fermented, cured and dried meats, fermented vegetables, ripened cheeses	Continental sausages/salamis, jerky, sauerkraut, olives, bean curd, cheddar, stilton, brie, fermented milk drinks and butter, yoghurt, etc	N/A	N/A	N/A
14. Cooked meat products that may be displayed for sale at ambient temperature for a limited period of time e.g. siu-mei and lo-mei	Chinese poached chickens, roasted ducks and roasted pork	<10 <sup>5</sup>	10 <sup>5</sup> -<10 <sup>6</sup>	≥10 <sup>6</sup>

N/A denotes "Not applicable"

Notes:

- a. For food items that are not included in these food categories, their ACC level should be interpreted taking into account the raw ingredients used, and the nature and degree of processing before sale.
- b. Most products are normally sterile when sampled from the container but if they are consumed after subsequent further preparation then assess them as Category 5.
- c. These products are 'Unsatisfactory' if spore forming anaerobes are present but these require special tests for detection and enumeration. Spore forming aerobes are also usually absent in foods that have been cooked in their container but low levels may occur in canned fish products.
- d. Check for signs of spoilage. Lactic acid bacteria can grow well at refrigeration temperatures and do not grow well aerobically. Spoilage will eventually occur at a level of around 10<sup>9</sup> cfu/g due to the production of lactic acid. If the predominant organisms are Gram-negative bacteria, spoilage is likely to be noticeable at 10<sup>7</sup> to 10<sup>8</sup> cfu/g, e.g. taints, discolouration, and slime produced by pseudomonads, slime produced by other Gram-negative bacteria.

## **Hygiene Indicator Organisms**

“Hygiene indicator organisms” refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food.

2. *E. coli* is a commonly used faecal indicator organism. Its presence in food generally indicates direct or indirect faecal contamination. Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage.

3. Enterobacteriaceae is a large group of biochemically and genetically related bacteria used to assess the general hygiene status of a food product. Their presence in heat treated food indicates inadequate cooking or post-processing contamination. In addition, some members of Enterobacteriaceae can contribute to the formation of histamine (scombrototoxin) in foods such as scombroid fish and occasionally some cheeses if these are not processed properly and/or stored at an adequate refrigeration temperature.<sup>1</sup>

4. Guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat food in general is provided in Table 1.3.

**Table 1.3 Guidance on the interpretation of results for hygiene indicator organisms in ready-to-eat food in general**

Hygiene indicator organism	Result (colony-forming unit (cfu)/g)		
	Satisfactory	Borderline	Unsatisfactory
Enterobacteriaceae <sup>a</sup>	<10 <sup>2</sup>	10 <sup>2</sup> - ≤10 <sup>4</sup>	> 10 <sup>4</sup>
<i>Escherichia coli</i> <sup>b</sup>	<20	20 - ≤10 <sup>2</sup>	> 10 <sup>2</sup>

Notes:

- a. The criterion listed for Enterobacteriaceae applies to heat treated food, fishes, and cheeses (excluding cheeses ripened using a culture of *Hafnia alvei* or *Proteus vulgaris*). It does not apply to fresh fruit and vegetables or food that contains fresh fruit and vegetables as ingredients e.g. sandwiches with salad, because these food types can contain high levels of Enterobacteriaceae as part of their normal micro-flora.
- b. Criterion does not apply to cheeses made from raw milk.

## Chapter II. Microbiological Criteria for Ready-to-eat Food in General – Specific Foodborne Pathogens

### Specific Foodborne Pathogens

Examination for foodborne pathogens (bacteria that may cause food poisoning) in ready-to-eat food contributes to food safety.

2. The symptoms of food poisoning vary from nausea and vomiting (e.g. caused by *Staphylococcus aureus* enterotoxin), through diarrhoea and dehydration (e.g. caused by *Salmonella* spp. and *Campylobacter* spp.) to severe conditions such as septicaemia, meningitis, paralysis and death (e.g. caused by invasive *Listeria monocytogenes* and in the rare cases of botulism caused by *Clostridium botulinum* toxin). The infective doses of different foodborne pathogens vary from less than ten to more than  $10^8$  organisms. General information on the common foodborne pathogens included in this set of guidelines is provided in Appendix I.

3. Guidance on the interpretation of results for specific foodborne pathogens in ready-to-eat food in general is provided in Table 2.1.

**Table 2.1 Guidance on the interpretation of results for specific foodborne pathogens in ready-to-eat food in general**

Criterion	Result (colony-forming unit (cfu)/g unless otherwise specified)		
	Satisfactory	Borderline	Unsatisfactory: potentially injurious to health and/or unfit for human consumption
<i>Campylobacter</i> spp. (thermotolerant)	n.d. in 25g	N/A	Detected in 25g
<i>Escherichia coli</i> O157 (and other Shiga toxin-producing <i>E. coli</i> (STEC))	n.d. in 25g	N/A	Detected in 25g
<i>Salmonella</i> spp.	n.d. in 25g	N/A	Detected in 25g
<i>Vibrio cholerae</i> (O1 and O139)	n.d. in 25g	N/A	Detected in 25g
<i>Shigella</i> spp. <sup>a</sup>	n.d. in 25g	N/A	Detected in 25g
<i>Listeria monocytogenes</i>			
● For refrigerated food <sup>b</sup> (excluding frozen food) or food intended for infants	n.d. in 25g <sup>c</sup>	N/A	Detected in 25g <sup>c</sup>
● For other ready-to-eat food	< 10 <sup>d</sup>	10 - ≤ 100 <sup>d</sup>	> 100 <sup>d</sup>
<i>Vibrio parahaemolyticus</i>	< 20	20 - ≤ 10 <sup>3</sup>	> 10 <sup>3</sup>
<i>Staphylococcus aureus</i> and other coagulase-positive staphylococci	< 20	20 - ≤ 10 <sup>4</sup>	> 10 <sup>4</sup>
<i>Clostridium perfringens</i>	< 10	10 - ≤ 10 <sup>4</sup>	> 10 <sup>4</sup>
<i>Bacillus cereus</i>	< 10 <sup>3</sup>	10 <sup>3</sup> - ≤ 10 <sup>5</sup>	> 10 <sup>5</sup>

n.d. = not detected; N/A = not applicable

Notes:

- Shigella* spp. would be tested in the cases of food poisoning investigation or food complaint if the organism is implicated but not recommended for routine surveillance.
- This criterion applies to all refrigerated food (excluding frozen food) unless there is scientific evidence supporting that the food concerned does not support the growth of *Listeria monocytogenes* under refrigeration. Reference can be made to the Codex Guidelines on the Application of General Principles of Food Hygiene to The Control of *Listeria monocytogenes* in Food (CAC/GL 61-2007).
- ISO 11290-1:1996/Amd 1:2004. Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

- d. ISO 11290-2:1998/Amd 1:2004. Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

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## Chapter III. Microbiological Criteria for Specific Food Items

### Introduction

This chapter contains microbiological criteria for specific food items, including ready-to-eat food.

2. The microbiological criteria for bottled waters, edible ice and non-bottled drinks were included in the Supplementary Information to Microbiological Guidelines for Ready-to-eat Food (February 2009), which were established and adopted by an Ad Hoc Working Group on Microbiological Safety of Food formed under the Expert Committee on Food Safety in 2008. These criteria were incorporated in this chapter while the microbiological criteria for natural mineral waters were revised in accordance with the latest version of the Codex Code of Hygienic Practice for Collecting, Processing and Marketing of Natural Mineral Waters (CAC/RCP 33-1985, revised 2011).<sup>2</sup> On the other hand, with consideration of local situation and expert opinion, the microbiological criteria for powdered formulae for infants and young children, treated, ready-to-eat spices as well as live or raw bivalve molluscs intended for direct consumption were adopted with reference to the respective Codex Code of Hygienic Practices and standard i.e. Code of Hygienic Practice for Powdered Formulae for Infants and Young Children (CAC/RCP 66-2008, revised 2009), Code of Hygienic Practice for Spices and Dried Aromatic Plants (CAC/RCP 42-1995), Standard for Live and Raw Bivalve Molluscs (CODEX STAN 292-2008, amendment 2013) and Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003, amendment 2013).<sup>3,4,5,6</sup>

3. Some food items that have been included in Chapter III (such as bottled waters, edible ice, non-bottled drinks, ready-to-eat spices and live or raw bivalve molluscs intended for direct consumption) also belong to ready-to-eat food. Hence, the microbiological criteria stipulated in Chapters I and II are applicable to these ready-to-eat food as well except for criteria that are laid down in this chapter.

### Definition

4. “Natural mineral water” is clearly distinguishable from ordinary drinking water. The former is characterised by its content of certain mineral

salts, trace elements or other constituents. Natural mineral water is collected directly from natural or drilled sources from underground water in which the original microbiological purity and chemical components are guaranteed. It is also packaged close to the point of emergence of the source under hygienic condition and not subjected to any treatment other than those permitted.<sup>7</sup>

5. “Non-bottled drinks” are classified as restricted food specified in Schedule 2 to the Food Business Regulation (Cap. 132X). Save with the permission of the Director of Food and Environmental Hygiene, no person shall sell non-bottled drinks. In this connection, a restricted food permit or a relevant permission on a food business licence is required for selling non-bottled drinks. Permittees / Licensees shall take all necessary steps to ensure that the non-bottled drinks are free from contamination. In order to monitor the hygiene condition under which the non-bottled drinks are prepared, there is a licensing condition on bacteriological standards for non-bottled drinks imposed on the concerned restricted food permits and food business licences.

6. “Infant formula” means a breast milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.<sup>3</sup>

7. “Follow-up formula” means a food intended for use as a liquid part of the weaning diet for the infant from the 6<sup>th</sup> month on and for young children.<sup>3</sup>

8. “Spices” include dried aromatic plants, relate to natural dried components or mixtures thereof, used in foods for flavouring, seasoning and imparting aroma. The term applies equally to spices in the whole, broken or ground form.<sup>4</sup>

9. “Live bivalve molluscs (intended for direct consumption)” are products that are alive immediately prior to direct consumption. Presentation includes the shell.<sup>5</sup>

10. “Raw bivalve molluscs (intended for direct consumption)” are products that were alive immediately prior to the commencement of processing for direct consumption. They have been shucked and/or frozen

and/or processed to reduce or limit target organisms while essentially retaining the sensory characteristics of live bivalve molluscs. Raw bivalve molluscs are marketed in a frozen or chilled state.<sup>5</sup>

### **Some Specific Microbiological Parameters Included in Chapter III**

11. Coliform bacteria can originate from faecal contamination or from the environment. Coliform bacteria are normally not present in natural mineral water sources. They are considered as an indicator of contamination of the water at source or during the packaging process.<sup>2</sup> In addition, coliform bacteria should be absent immediately after disinfection, and the presence of these organisms in water indicates inadequate treatment.

12. Enterococci are a sub-group of faecal streptococci. Compared to *E. coli* and coliforms, they tend to survive longer in the water environment and are therefore used as an additional indicator of faecal contamination.<sup>2</sup>

13. The spores of spore-forming sulphite-reducing anaerobes are very resistant towards various kinds of environmental stresses. These bacteria can originate from faecal contamination and due to the length of their survival in unfavourable environments, they are usually used as an indicator of faecal contamination.<sup>2</sup>

14. *Pseudomonas aeruginosa* is a common environmental microorganism and can be found in faeces, soil, water and sewage. It can multiply in water environments and also on the surface of suitable organic materials in contact with water. *Pseudomonas aeruginosa* is not a normal component of the natural flora of natural mineral waters. Its presence is considered as an indicator of contamination of the water at source or during the packaging process.<sup>2</sup>

15. *Enterobacter sakazakii* (*Cronobacter* spp.) is a pathogen that generally causes disease only in people with weakened immune systems. The bacterium can cause invasive infections (e.g. sepsis or meningitis) in infant. Neonates ( $\leq 28$  days old) and infants less than 2 months of age, in particular those that are pre-term, low-birth-weight ( $<2.5$  kg) and immunocompromised, are at greatest risk. Powdered infant formulae were established as the source of *E. sakazakii* (*Cronobacter* spp.).<sup>3</sup>

## **Interpretation of Microbiological Results**

16. Any food samples failing any of the microbiological criteria stipulated in this chapter will be considered as “Unsatisfactory: Potentially injurious to health and/or unfit for human consumption”. In other words, the affected products should be prevented from being released for human consumption. In such cases, appropriate actions (not exclusive) should be taken i.e. immediate investigation; parties concerned (e.g. vendors) should be instructed to stop sale of food item in question, investigate immediately and find out the causes and to adopt measures to improve the situation. Take investigative samples. In addition, warning letters, source tracing and other enforcement actions should be considered.

## 1. Microbiological Criteria for Bottled Waters

Table 3.1 Microbiological criteria for natural mineral waters

<b>Parameters</b>	<b>n</b>	<b>c</b>	<b>m</b>	<b>Method*</b>
<i>E. coli</i>	5	0	n.d. in 250ml	ISO 9308-1
Coliform bacteria	5	0	n.d. in 250ml	ISO 9308-1
Enterococci	5	0	n.d. in 250ml	ISO 7899-2
Spore-forming sulphite reducing anaerobes	5	0	n.d. in 50ml	ISO 6461-2
<i>Pseudomonas aeruginosa</i>	5	0	n.d. in 250ml	ISO 16266:2006

Where 'n' = number of samples that must conform to the criteria; 'c' = the maximum allowable number of defective sample units in a 2-class plan; 'm' = a microbiological limit which, in a 2-class plan separates good quality from defective quality.

\* Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

n.d. = not detected

Table 3.2 Microbiological criteria for bottled/packaged drinking waters (other than natural mineral waters)

<b>Parameters</b>	<b>Limits</b>
<i>E. coli</i>	n.d. in 100ml
Coliform bacteria	n.d. in 100ml
<i>Pseudomonas aeruginosa</i>	n.d. in 250ml

n.d. = not detected

## 2. Microbiological Criteria for Edible Ice

Table 3.3 Microbiological criteria for ice from ice manufacturing plants and packaged ice from retail outlets

<b>Parameters</b>	<b>Limits</b>
ACC [37°C/48 hours]	<500 cfu/ml
<i>E. coli</i>	n.d. in 100ml
Coliform bacteria	n.d. in 100ml

cfu = colony-forming unit

n.d. = not detected

Table 3.4 Microbiological criteria for loose ice from retail outlets

<b>Parameters</b>	<b>Limits</b>
ACC [37°C/48 hours]	<1,000 cfu/ml
<i>E. coli</i>	n.d. in 100ml
Coliform bacteria	<100 cfu/100ml

cfu = colony-forming unit

n.d. = not detected

## 3. Microbiological Criteria for Non-bottled Drinks

These criteria are included in the licensing condition for non-bottled drinks.

Table 3.5 Microbiological criteria for non-bottled drinks

<b>Parameters</b>	<b>Limits</b>
<i>E. coli</i>	<100 cfu/ml
<i>Salmonella</i> spp.	n.d. in 25ml
<i>Staphylococcus aureus</i>	<100 cfu/ml
<i>Clostridium perfringens</i>	<100 cfu/ml

cfu = colony-forming unit

n.d. = not detected

#### **4. Microbiological Criteria for Powdered Formulae for Infants and Young Children**

These products are to be distinguished from ready-to-feed liquid formulae. Ready-to-feed liquid formulae for infants and young children shall be a commercially sterile product.

Table 3.6 Microbiological criteria for powdered infant formulae and formulae for special medical purposes† and human milk fortifiers

<b>Microorganisms</b>	<b>n</b>	<b>c</b>	<b>m</b>	<b>Method*</b>
<i>Enterobacter sakazakii</i> ( <i>Cronobacter</i> spp.)	30	0	n.d. in 10g	ISO/TS 22964:2006
<i>Salmonella</i> spp.	60	0	n.d. in 25g	ISO 6579

† This category includes formula for special medical purposes intended for infants as the sole source of nutrition and formula for special medical purposes for infants, intended to partially replace or supplement breast-milk or infant formula.

Where ‘n’ = number of samples that must conform to the criteria; ‘c’ = the maximum allowable number of defective sample units in a 2-class plan; ‘m’ = a microbiological limit which, in a 2-class plan separates good quality from defective quality.

\* Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

n.d. = not detected

Table 3.7 Microbiological criterion for powdered follow-up formulae and formulae for special medical purposes for young children

<b>Microorganisms</b>	<b>n</b>	<b>c</b>	<b>m</b>	<b>Method*</b>
<i>Salmonella</i> spp.	60	0	n.d. in 25g	ISO 6579

Where ‘n’ = number of samples that must conform to the criteria; ‘c’ = the maximum allowable number of defective sample units in a 2-class plan; ‘m’ = a microbiological limit which, in a 2-class plan separates good quality from defective quality.

\* Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

n.d. = not detected

## **5. Microbiological Criterion for Treated, Ready-to-eat Spices**

Table 3.8 Microbiological criterion for treated, ready-to-eat spices

<b>Microorganisms</b>	<b>n</b>	<b>c</b>	<b>m</b>
<i>Salmonella</i> spp.	10	0	n.d. in 25g

Where 'n' = number of samples that must conform to the criteria; 'c' = the maximum allowable number of defective sample units in a 2-class plan; 'm' = a microbiological limit which, in a 2-class plan separates good quality from defective quality.

n.d. = not detected

## **6. Microbiological criteria for live or raw bivalve molluscs intended for direct consumption**

These criteria do not apply to scallops when the final product is the adductor muscle only.

Table 3.9 *E. coli* criterion for live or raw bivalve molluscs

<b>Microorganisms</b>	<b>n</b>	<b>c</b>	<b>m</b>	<b>M</b>	<b>Method*</b>
<i>E. coli</i>	5	1	230 MPN/100g	700 MPN/100g	ISO 16649-3

Where 'n' = the number of sample units; 'c' = the number of sample units that may exceed the limit 'm', and 'M' is the limit which no sample unit may exceed.

\* Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated.

MPN = most probable number

Table 3.10 *Salmonella* spp. criterion† for live or raw bivalve molluscs

<b>Microorganisms</b>	<b>n</b>	<b>c</b>	<b>m</b>
<i>Salmonella</i> spp.	5	0	n.d. in 25g

† This criterion will not be included in routine surveillance unless for products with considerable manual handling.

Where 'n' = number of samples that must conform to the criteria; 'c' = the maximum allowable number of defective sample units in a 2-class plan; 'm' = a microbiological limit which, in a 2-class plan separates good quality from defective quality.

n.d. = not detected

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## Appendix I Common Foodborne Pathogens

This appendix provides general information on the common foodborne pathogens included in this set of guidelines for reference.

**Table A1 Summary on common foodborne pathogens included in this set of guidelines - infective dose, incubation period and associated foods**

Common foodborne pathogens	Infective dose*	Incubation period**	Associated foods**
<i>Bacillus cereus</i> ◇	Greater than 10 <sup>6</sup> organisms per gram in food is indicative of a potential human health hazard; the number of organisms most often associated with human illness is 10 <sup>5</sup> to 10 <sup>8</sup> ; however, the pathogenicity arises from preformed toxin	Emetic intoxication (Preformed heat stable toxin in food): Usually 1 – 6 hours	Meat, stews, gravies and improperly refrigerated cooked and fried rice
		Diarrhoeal (Toxin produced inside body): Usually 10 – 16 hours	
<i>Campylobacter</i> spp.	About 10 <sup>4</sup> organisms in general; however, in trials, as few as 500 organisms led to disease in some individuals	Usually 2 – 5 days ( <i>C. jejuni</i> )	Raw and undercooked poultry
<i>Clostridium perfringens</i> ◇	Greater than 10 <sup>6</sup> organisms or greater than 10 <sup>6</sup> spores per gram of food; toxin production in the digestive tract is associated with sporulation	Range from 6-24 hours; usually 10-12 hours	Meat, poultry and gravies
<i>Escherichia coli</i> O157 (and other Shiga toxin-producing <i>E. coli</i> (STEC))	As low as 10 organisms ( <i>E. coli</i> O157:H7)	Range from 2 – 10 days; usually 3 - 4 days	Raw or undercooked ground meat products, fruits and vegetables
<i>Listeria monocytogenes</i>	Undetermined; less than 10 <sup>3</sup> organisms may cause disease in susceptible individuals	Range from 3 – 70 days; 3 weeks on average	Ready-to-eat food with long shelf lives under refrigeration e.g. soft cheese and cold cuts
<i>Salmonella</i> spp.	Typhoid fever: less than 10 <sup>3</sup> organisms	Range from 7 – 21days	Food, water or beverages contaminated with faeces and urine of infected people e.g. shellfish (particularly oysters), raw fruits and vegetables and unpasteurised milk and dairy products

	Nontyphoidal Salmonellosis: As few as 1 organism	Range from 6 – 72 hours; usually about 12 – 36 hours	Inadequately cooked meat and poultry products; contaminated raw eggs, egg products, fruits and vegetables; and unpasteurised dairy products
<i>Shigella</i> spp.	As few as 10 organisms	Usually 1 – 3 days, but can be up to 7 days	Contaminated raw food e.g. salads and sandwiches
<i>Staphylococcus aureus</i>	Less than 1µg preformed heat stable toxin ; greater than 10 <sup>5</sup> organisms per gram in food is needed to produce this toxin level	Range from 30 minutes to 8 hours; usually 2 – 4 hours	Any food contaminated by food handlers with skin infection or nasal carriers, especially those food involving manual handling and no reheating afterwards e.g. sandwiches, cakes and pastries
<i>Vibrio parahaemolyticus</i>	Risk assessment states that the median infective dose is 10 <sup>8</sup> organisms; however, evidence from an outbreak suggests an infectious dose >1,000-fold less than that	Usually 12 – 24 hours	Seafood, salted food e.g. salted vegetables and smoked knuckles or other food cross-contaminated by seafood
<i>Vibrio cholerae</i>	About 10 <sup>6</sup> organisms	Range from a few hours to 5 days, usually 2 – 3 days	Contaminated fish and shellfish

\* Adapted from Bad Bug Book 2<sup>nd</sup> edition: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook of U.S. FDA. [Accessed 2 May 2013].

\*\* Adapted from (1) Diagnosis and Management of Foodborne Illnesses – A Primer for Physicians and Other Health Care Professionals: Foodborne Illnesses Table: Bacterial Agents. AMA/CDC/FDA/US Department of Agriculture. February 2004. and (2) Communicable Diseases of Centre for Health Protection. [Accessed 2 May 2013]

◇ Spore forming

### ***Bacillus cereus***

2. *Bacillus cereus* is a spore-forming bacterium ubiquitous in the environment. It is readily isolated from soil, cereal crops and vegetables etc. It has been reported that soil can contain approximately 1,000 to 100,000 spores per gram.<sup>1</sup> Hence, it is not uncommon to find this bacterium in food, especially in raw agricultural products such as raw fruit and vegetables, raw herbs. These foods usually contain less than 100 spores per gram, but higher amount may be found in some herbs and spices.<sup>1</sup>

3. *B. cereus* can form spores which are able to resist heat and survive the cooking temperature. It can either grow in the presence or absence of oxygen. The optimal growth temperature for *B. cereus* is around 30°C to 37°C.<sup>1</sup> At temperature below 10°C, *B. cereus* is unable to grow and produce toxin that causes vomiting.<sup>1</sup> Therefore, controlling

storage temperature of food is important to prevent foodborne disease caused by this bacterium.

4. There are two types of food poisoning caused by different toxins produced by *B. cereus*. Emetic (cause vomiting) intoxication is caused by a heat-stable toxin (which can resist 126°C for 90 minutes) preformed in food.<sup>1</sup> Symptoms including nausea and vomiting occur in the first few hours after ingestion of incriminated food, followed by diarrhoea in some cases. Another type of poisoning is diarrhoeal, which is characterised by watery diarrhoea associated with abdominal pain. This type resembles the illness caused by *Clostridium perfringens* in which the toxins are produced in the intestine by ingested spores or vegetative cells. The illnesses of these two types of food poisoning are generally mild and persist no longer than 24 hours.<sup>2,3</sup>

#### ***Campylobacter* spp.**

5. *Campylobacter* spp. contain different species and subspecies, of which the most frequently reported in human disease is *C. jejuni*, and less commonly *C. coli*.<sup>4,5,6</sup> These and the other campylobacters are widely distributed in most warm-blooded animals such as poultry, cattle, pigs, sheep and dogs.<sup>5</sup> *C. jejuni* has a very varied reservoir but is predominantly associated with poultry. *C. coli* is predominantly found in pigs.<sup>7</sup>

6. Many disease-causing *Campylobacter* spp. can tolerate higher growth temperature. *C. jejuni* and *C. coli* are distinguished from most other *Campylobacter* spp. by their high optimum growth temperature (42°C).<sup>7</sup> On the other hand, most *Campylobacter* spp. prefer a micro-aerobic atmosphere (containing 3-10% oxygen) for growth.<sup>5</sup>

7. The most common symptoms of *Campylobacter* infection include diarrhoea, which may be watery or sticky and can contain blood, abdominal pain, fever, headache, nausea, and/or vomiting.<sup>5</sup> Food poisoning caused by *C. jejuni* generally lasts for 2-10 days.<sup>2</sup>

#### ***Clostridium perfringens***

8. *C. perfringens* is widely distributed in the environment and frequently found in intestines of both humans and animals, hence is likely to be present in foods of animal origin and vegetables exposed to soil, dust or faecal material. The organism is not uncommonly found in food, particularly gravies.<sup>8</sup>

9. The organism is a spore-forming bacterium, which is able to form heat resistant spores under unfavourable condition, e.g. limited nutrient availability. Although the bacterium is an anaerobe, which grows in the absence of oxygen, it can tolerate low oxygen level for some time. The organism is characterised by their high optimum growth temperature at 43°C, but unable to grow at temperature lower than 10°C. Some strains can grow very fast with a doubling time of less than ten minutes.<sup>8</sup>

10. Heat of cooking can activate the germination of *C. perfringens* spores which survive in anaerobic conditions like inside internal cavities, rolls of meat, stuffed poultry, or gravies. The organism can then multiply in the area where oxygen level is low; cooling of food at ambient temperature for a long period allows rapid multiplication of the bacterium.<sup>8</sup> Intake of the food containing large number of the organisms allows sufficient amount to survive the passage through stomach, which subsequently form spores accompanied with toxin in the intestine.<sup>2</sup> Hence, foods prepared in bulk, especially cooked meat and poultry dishes, and stored at ambient temperatures with a long cooling period after cooking are at high risk.

11. *C. perfringens* poisoning is usually characterised by sudden onset of abdominal pain followed by diarrhoea and nausea.<sup>9</sup> The illness is usually over within 24 hours but less severe symptoms may persist in some individuals for 1 or 2 weeks. A few deaths have been reported as a result of dehydration and other complications.<sup>2</sup>

#### ***Escherichia coli* O157 (and other Shiga toxin-producing *E. coli* (STEC))**

12. Shiga toxin-producing *E. coli* (STEC) is a group of *E. coli* that produces one or more verocytotoxins (VT), also known as Shiga-like toxins. This group of bacteria is also called Verocytotoxin-producing *E. coli* (VTEC).<sup>10</sup> STEC is transmitted to humans primarily through consumption of contaminated foods, such as raw or uncooked ground meat products, contaminated fruits and vegetables and direct contact with animals and their environment. Direct person-to-person transmission through the oral-faecal route can also occur.

13. *E. coli* O157:H7 is the predominant serotype in a pathogenic subset of STEC, designated enterohaemorrhagic *E. coli* (EHEC). The designation is based on their capacity to cause attaching and effacing lesions in epithelial cells of intestine, and their ability to cause haemorrhagic colitis (bloody diarrhoea) and a life-threatening complication haemolytic uraemic syndrome (HUS) in humans. Other non-O157 serogroups, including

O26, O91, O103, O104, O111, O113, O117, O118, O121, O128 and O145, have been associated with occasional outbreaks of human disease, and others may be associated with sporadic cases.<sup>11</sup>

14. The illness caused by STEC infection usually present with diarrhoea, often bloody diarrhoea, abdominal cramps and vomiting. In serious cases, the infection may lead to HUS which is a type of kidney failure. Symptoms of HUS vary, depending on the patient's health and the extent of the infection. People of any age can become infected. Very young children and the elderly are more likely to develop severe illness, but even healthy older children and young adults can become seriously ill.<sup>10</sup>

15. In general, the non-O157 serogroup is less likely to cause severe illness than *E. coli* O157:H7; however, some non-O157 STEC serogroups can cause the most severe manifestations of STEC illness.<sup>10</sup>

### ***Listeria monocytogenes***

16. *Listeria monocytogenes* is universally found in the environment, particularly in soil, vegetation, animal feed, and in human and animal faeces. Such bacterium can survive and multiply at temperature as low as 0°C, but can be easily destroyed under normal cooking temperature. Conditions with pH that ranges from 4.4-9.4 and water activity that equals or is greater than 0.92 may support the growth of *L. monocytogenes*.<sup>12</sup> However, a combination of factors (pH, water activity) can also control the growth of *L. monocytogenes* in foods.<sup>13</sup> Consuming *Listeria* contaminated food may lead to the development of a disease called listeriosis.

17. Ready-to-eat foods with long shelf lives under refrigeration such as soft cheeses and ready-to-eat poultry and meat pose the greatest risk as *L. monocytogenes* may grow to significant numbers at refrigeration temperatures when given sufficient time.<sup>14,15</sup> However, the growth of *L. monocytogenes* is not supported under freezing condition.

18. Foodborne listeriosis is a relatively rare but serious disease with high fatality rates (20%–30%).<sup>12</sup> *Listeria* predominantly affects foetuses and newborns, elderly and immunocompromised individuals such as patients with AIDS, diabetes mellitus or cancer.<sup>16</sup> A person with listeriosis usually presents with fever, headache and sometimes gastrointestinal symptoms such as nausea, vomiting and diarrhoea. Asymptomatic infection probably occurs in most people. However, serious infections of *L. monocytogenes* are manifested by meningitis and septicemia.<sup>17</sup> Even though symptoms may be relatively

mild in mothers, the passage of *L. monocytogenes* through the placenta may cause miscarriage or stillbirth or her newborn resulting in septicaemia and meningitis.<sup>2</sup>

### ***Salmonella* spp.**

19. Salmonellae are bacteria found in the intestinal tract of man and animals. More than 2,500 serotypes of salmonellae have been identified and *Salmonella* Enteritidis, followed by *Salmonella* Typhimurium, are the most commonly isolated serotypes in Hong Kong.

20. Food may be contaminated by salmonellae in animal faeces and cross-contamination may occur during further processing and preparation. Salmonellae may survive in the environment and equipment of food-processing facilities. Salmonellae reside in the intestinal tract and are shed in the faeces of infected animals and humans as well. Many foods, particularly those of animal origin and those subject to sewage pollution, have been identified as vehicles for transmitting these pathogens.<sup>18</sup>

21. Poultry and poultry products are commonly linked to *Salmonella* and the bacterium can also be found inside the eggs. Eggs may be contaminated via two different routes: vertical transmission through the ovary or transovarian or horizontal transmission through the shell or trans-shell.<sup>19</sup> Through vertical transmission, bacteria are introduced from infected reproductive tissues to eggs prior to shell formation. Horizontal transmission usually occurs from faecal contamination on the egg shell as the eggs are released via the cloaca, where the excretion of faeces also takes place. It also includes contamination through environmental vectors, such as farmers, pets and rodents. Under appropriate conditions, bacteria on the shell can move across shell into the egg content.<sup>20</sup>

22. Enteric fever (also known as typhoid fever) is an illness caused by *S. Typhi* and *S. Paratyphi* type A, B and C.<sup>21</sup> Symptoms of typhoid fever include high fever, diarrhoea or constipation, headache and sometimes a rash.<sup>2</sup> It can be complicated by intestinal bleeding and perforation, impaired consciousness and even death if untreated. On the other hand, symptoms of nontyphoidal salmonellosis, which is caused by serotypes other than *S. Typhi* and *S. Paratyphi*, include nausea, vomiting, abdominal cramps, diarrhoea, fever, headache.<sup>2</sup>

### ***Shigella* spp.**

23. *Shigella* bacteria are found naturally in the intestinal tracts of humans and other primates. People who eat food or drink water contaminated by *Shigella* can become ill

with bacillary dysentery (shigellosis). In addition, the bacteria may spread from person to person by physical contact. Contamination through food handler is one of the major sources, and food can also become contaminated by flies carrying sewage or faeces.

24. Severity of illnesses varies with the host and the type of *Shigella*. The illness is characterised by sudden onset of fever, diarrhoea with abdominal cramps and nausea or vomiting. The stool may contain blood and mucus (dysentery). Mild and asymptomatic illness can occur. Complications include toxic dilation of large intestine and acute kidney disease. *Shigella dysenteriae* type 1 is of particular concern in developing countries, where it spreads in epidemics and is often associated with serious disease and complications. The case-fatality rates have been as high as 20% among hospitalised cases.<sup>22</sup> Locally, the most common species isolated from patients is *S. sonnei*.<sup>23</sup>

### ***Staphylococcus aureus***

25. *Staphylococcus aureus* is a bacterium which is commonly present in human nasal passage, throat, hair and skin without causing any discomfort. It has the ability to produce several enterotoxins that are responsible for food poisoning. The temperature range for the bacterium to form toxin is from 10 to 45°C and optimal at around 35 to 40°C. Hence, normal refrigeration temperature can restrict the formation of toxin. On the other hand, *S. aureus* is a salt-tolerant microorganism and grows at a water activity as low as 0.85 which corresponds to a salt content around 25% w/w. Hence, it may grow better than the other bacteria in salt-containing products or products with low water activities.<sup>24</sup>

26. Even though most cases of infection are caused by *S. aureus*, other coagulase-positive *Staphylococcus* species, such as *S. intermedius* can also produce enterotoxins that cause food poisoning.<sup>25</sup> The bacterium can be destroyed by normal cooking procedures or pasteurisation, while the toxins produced are more resistant to heat; they may survive in food causing food poisoning.<sup>24</sup>

27. The most common way of contamination of food is by contact with food handlers' hands, especially in the cases where the food is handled subsequent to cooking. Prolonged storage without refrigeration allows the bacteria to grow and form toxins. Since the toxins are heat stable, the incriminated food may also cause food poisoning even if it is further heat treated.

28. The main symptoms of *Staphylococcus aureus* poisoning are nausea, vomiting, retching, abdominal cramping and prostration, often accompanied by diarrhoea and

sometimes fever. In severe cases, patients may present with headache, muscle cramping, severe fluid and electrolytes loss with weakness and low blood pressure or shock. Patients usually recover within two days, but can take longer in severe cases that may require hospitalisation.<sup>26</sup>

### ***Vibrio parahaemolyticus***

29. *Vibrio parahaemolyticus*, similar to other *Vibrio* spp., occurs naturally in marine, coastal, and estuarine (brackish) environments. The bacterium is characterised by its rapid growth under favourable conditions.<sup>27</sup>

30. Raw, partially treated, and recontaminated seafood products have been associated with the foodborne illnesses caused by *V. parahaemolyticus*. These seafood or seafood products include crayfish, lobster, shrimp, fish-balls, boiled surf clams, fried mackerel, mussel, tuna, seafood salad, raw oysters and clams.<sup>27</sup>

31. In addition, seafood products without proper storage during summer months are of higher risk. A correlation exists between the probability of infection and warmer months of the year.<sup>2</sup> Contaminated *V. parahaemolyticus* will multiply on seafood products without proper refrigeration, which increases the possibility of infection.

32. Symptoms of *V. parahaemolyticus* infections include diarrhoea, abdominal cramps, nausea, vomiting, fever and bloody diarrhoea. Most cases are self-limiting, however, severe cases requiring hospitalisation have been reported.<sup>2</sup>

### ***Vibrio cholerae***

33. *V. cholerae*, unlike most other *Vibrio* spp, can survive in freshwater environment. It is indigenous to fresh and brackish water environments worldwide. Among more than 200 O serogroups of *V. cholerae*, strains belonging to O1 and O139 serotypes generally possess the *ctx* gene and produce cholera toxin (CT) and are responsible for epidemic cholera. Ingested bacteria can attach to the small intestine and produce CT which results in the watery diarrhoea associated with cholera. Epidemic cholera is confined mainly to developing countries with warm climates. Some strains belonging to the O serogroups other than O1 and O139 (referred as non-O1/non-O139) can cause foodborne diarrhoea that is milder than cholera.<sup>27</sup>

34. Cholera is exclusively a human disease, in which contamination by faeces from

infected individual is a major cause of infection in cholera epidemics. Contamination of food production environments (including aquaculture ponds) by faeces can indirectly introduce the bacterium into foods. Seafood, including bivalve molluscs, crustaceans, and finfish, are most often incriminated in foodborne cholera cases in many countries.<sup>27</sup>

35. Common symptoms of cholera may vary from a mild, watery diarrhoea to an acute diarrhoea, with characteristic rice water stools. Other symptoms include vomiting and dehydration; after severe fluid and electrolyte loss, death may occur.<sup>2</sup>

#### **For further information:-**

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## References to Appendix I

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## **Appendix II: Guidance Notes on Sampling Plan for Microbiological Analysis**

### **Sampling plan**

Sampling plan is a systematic way to assess the microbiological quality of food lots. A “lot” refers to a batch of products manufactured under the same conditions at the same time. During sampling, the samples should be taken from the lot independently and randomly.

In developing a sampling plan, a number of factors should be taken into consideration including properties of food, production processes, storage conditions of the final products, associated risks, targeted consumers and practical limitations. Each food product should be considered individually.

A comprehensive sampling plan includes the following elements:

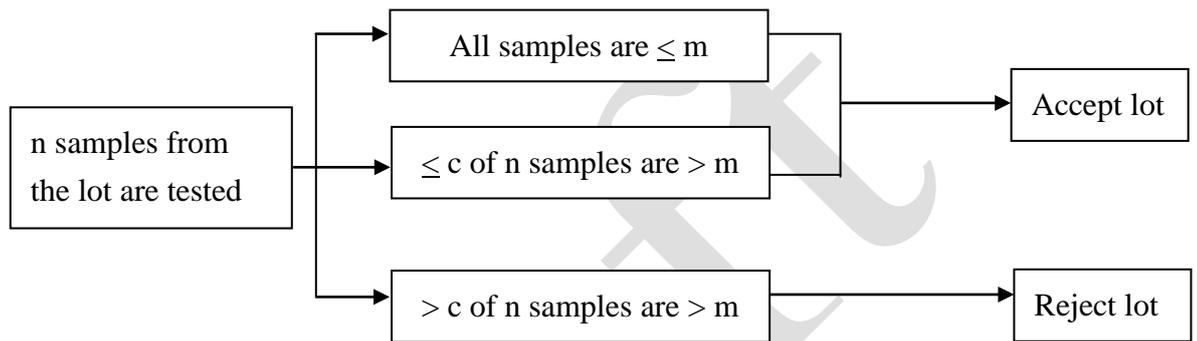
- (a) The microbe or group of microbes of concern or interest;
- (b) Number of samples to be tested (n);
- (c) Testing method(s);
- (d) Microbiological limit(s), m & M
  - Acceptable ( $\leq m$ )
  - Marginally acceptable ( $> m$  and  $\leq M$ )
  - Unacceptable ( $> M$ );
- (e) Number of samples which fall into each category of microbiological limit (i.e. acceptable / marginally acceptable / unacceptable).

### **Types of sampling plan**

Two types of sampling plans are commonly used in food microbiology, namely, the two-class attributes plan and the three-class attributes plan.

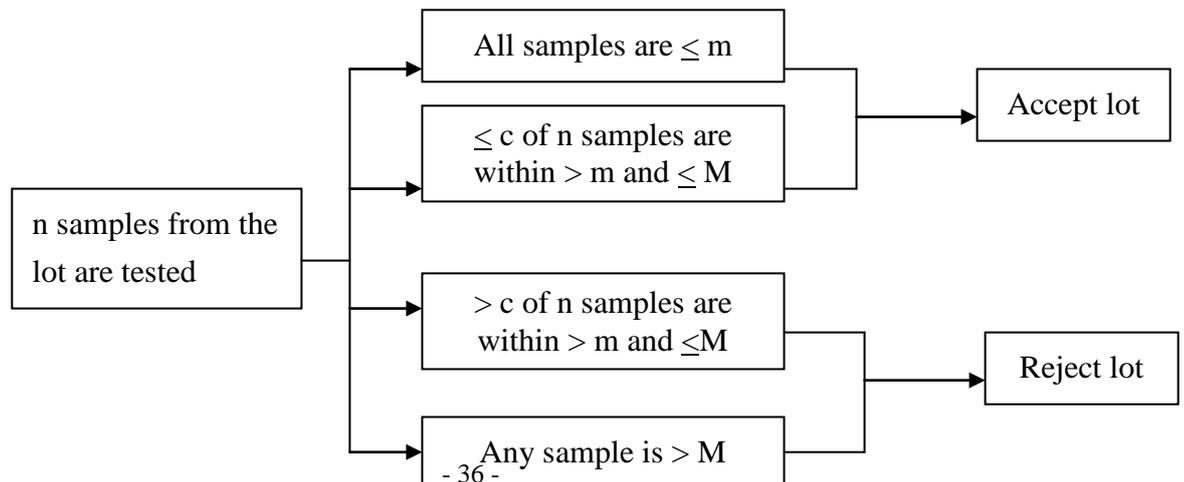
*Two-class attributes plan:*

Under this plan, sample(s) is (are) taken from the lot and tested. As only one microbiological limit “m” is involved in this plan, therefore two classes of attributes,  $\leq m$  &  $> m$ , could be identified. The maximum allowable number of sample(s) that yielded unsatisfactory test results is represented by “c”. The lot will be accepted or rejected as illustrated in the following diagram:



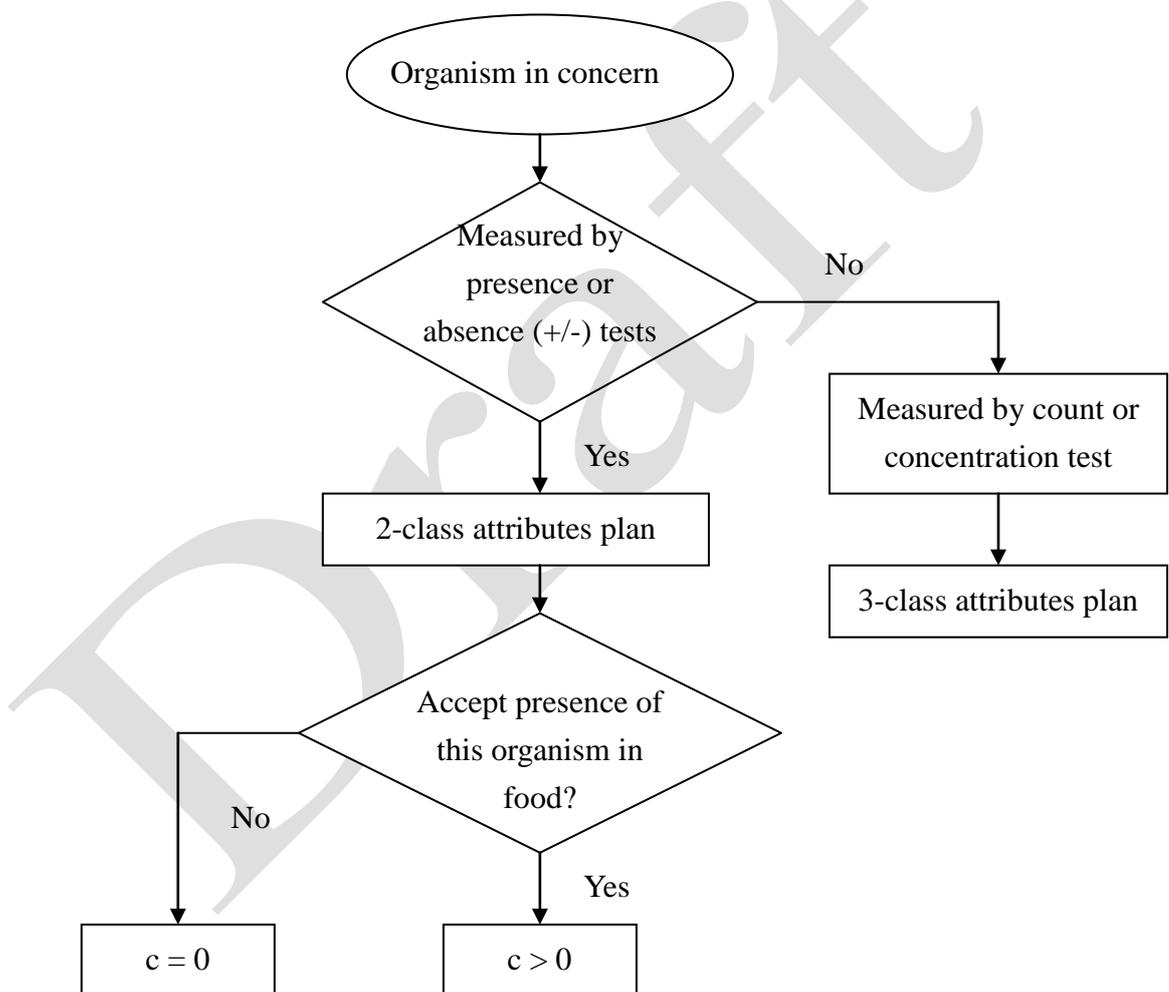
*Three-class attributes plan:*

For a three-class attributes plan, two microbiological limits, m & M, are set. The microbiological limit “m” commonly reflects the upper limit of a good manufacturing practice (GMP). The criterion “M” marks the limit beyond which the level of contamination is hazardous or unacceptable. The lot will be accepted or rejected as shown in the following diagram:



## Choice of sampling plan

In general, a two-class attributes plan is preferred when the organism of concern is not permitted in food sample. If the number of microbes in a unit-volume is allowable, a three-class attributes plan is usually adopted. The following decision tree shows how to choose an appropriate sampling plan for a specific application.



To enhance food safety and improve food quality, more stringent microbiological limits (by decreasing values of m and/or M) should be adopted. By changing the value(s) of c and/or n, the stringency of sampling plan can also be adjusted.

### **International development of sampling plan**

In 1981, the Codex Alimentarius Commission adopted the generic approach on sampling plan developed by the International Committee of Microbiological Specification for Foods (ICMSF). The ICMSF's sampling plan is recommended and used by the international bodies, food authorities in some countries and some international food manufacturers.

For details of the principle and application of ICMSF's sampling plan, please refer to the ICMSF publication - *Microorganisms in Foods 2, Sampling for Microbiological Analysis: Principles and Specific Applications* (2<sup>nd</sup> edition; 1986) and *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (2002).

**Remarks: Food samples are regularly taken by the Centre for Food Safety (CFS) for microbiological analysis. The presence of microorganisms found in any of the food samples taken is not allowed to exceed the microbiological limits as prescribed in the legislation nor should it exceed the microbiological guideline levels as adopted by CFS. The food trade may adopt a suitable sampling plan as discussed in the "Guidance Notes on Sampling Plan for Microbiological Analysis" to monitor the safety and quality of their food products.**