

## Microbiological Guidelines for Ready-to-eat Food

### Supplementary Information to Microbiological Guidelines for Ready-to-eat Food

#### 1. What are the existing microbiological standards and guidelines stipulated in the Hong Kong Special Administrative Region?

The relevant food safety Regulation is laid down in the Public Health and Municipal Services Ordinance (PHMSO), Chapter 132. Section 54 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 the food is not of the nature, substance, quality of the food demanded by the purchaser. In addition, specific legal microbiological standards for some specified foods e.g. **frozen confections** and **milk** are also stipulated in the relevant subsidiary legislations. For example:

##### (a) Frozen Confections Regulation

- |  |           |
|--|-----------|
| <input type="checkbox"/> Aerobic plate count (APC):  | ≤50,000/g |
| <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Coliform count: | ≤100/g    |

##### (b) Milk Regulation

- |   |                |                   |
|---|----------------|-------------------|
| <input type="checkbox"/> Raw milk (before heat-Treatment) | APC            | ≤200,000/ml       |
|   | Coliform count | absent in 0.001ml |
| <input type="checkbox"/> Pasteurized milk                 | APC            | ≤30,000/ml        |
| <input type="checkbox"/>                                  | Coliform count | absent in 0.1ml   |
| <input type="checkbox"/> Sterilized milk                  | Colony count   | < 10              |

A set of Microbiological Guidelines for Ready-to-eat Food (the Guidelines) has also been developed, stipulating the microbiological guidelines for ready-to-eat food. The Guidelines aim at assisting government officials and the trade to check and monitor the safety of ready-to-eat food. The trade has to ensure that any food intended for human consumption is fit and in compliance with the microbiological limits as prescribed in the relevant legislations. The trade should also refer to the limits set in the Guidelines so as to ensure safety and hygienic quality of ready-to-eat food.

#### 2. How are the Guidelines developed?

In 2002, under the advice of the Expert Panel on Microbiological Safety of Food (the Panel), an expert group set up to advise the Director of Food and Environmental Hygiene, a set of microbiological guidelines for ready-to-eat food was developed by the Food and Environmental Hygiene Department. In light of changing needs and latest expert views, the Guidelines have been revised in 2007 by the Centre for Food Safety, making reference to international practice and in consultation with the Panel.

#### 3. What is the purpose for establishing the Microbiological Guidelines for Ready-to-eat Food?

The purpose of the Guidelines is to provide assistance to officers in the interpretation of microbiological analyses of ready-to-eat food and recommendations on the appropriate follow-up action for food safety monitoring and control. The food trade can make use of the Guidelines to formulate design requirements and to examine end-products as one of the measures to verify or validate efficacy of the food safety production plan.

#### 4. What are the major microbiological components listed in the Guidelines?

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

“Aerobic colony count (ACC)” is a count of viable bacteria based on counting of colonies grown in nutrient agar plate at **30°C for 48 hours**. ACC is useful for indicating the sanitary quality of food. Generally, it does not relate to food safety hazards, but is taken as a food quality parameter.

### **Indicator organism - *Escherichia coli* (total) count**

“Indicator organisms” refer to the selected surrogate markers employed to reflect the hygienic quality of food. *E. coli* is commonly used as surrogate indicator. The native habitat for *E. coli* is the enteric tract of human and animals. 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. The presence of *E. coli* in foods does not connote directly the presence of a pathogen, but implies a certain risk that it may be present.

### **Specific pathogens**

“Specific pathogens” refer to bacteria that may cause food poisoning. Mechanisms involved may be toxins produced in food or intestinal infection. Symptoms of food poisoning vary from nausea and vomiting (e.g. caused by *S. aureus*), through diarrhoea and dehydration (*Salmonella* spp. and *Campylobacter* spp.) to paralysis and death in the rare cases of botulism. The infectious doses vary from less than 10 to more than 10<sup>6</sup> organisms.

The Guidelines stipulate the safety limits of nine major foodborne pathogens in ready-to-eat foods, making reference from scientific information, expert advice and international practices. A “not detected in 25g” limit is set for *Campylobacter* spp., *Escherichia coli* O157:H7, *Salmonella* spp. and *Vibrio cholerae* in all ready-to-eat food. Satisfactory limit ranging from <20-1000 colony-forming units per gram (cfu/g) is set for *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus*. Two limits are set for *Listeria monocytogenes* depending on the storage conditions/ targeted consumers of ready-to-eat foods.

### **5. For assessment of hygienic quality, food items are grouped into five categories. How are the food items classified?**

In the Guidelines, food items are grouped into five categories for the assessment of hygienic quality, taking into account the raw ingredients used, and the nature and degree of processing before sale. The categorisation is summarised in the Food Category Table for Aerobic Colony Count Assessment. The same principle for categorisation is applicable to other ready-to-eat food that is not listed in the table.

### **6. What will be the action taken by the authority if the microbiological quality of a food sample is classified as unsatisfactory or unacceptable?**

If the microbiological status of a food sample is unsatisfactory (sub-optimal hygienic conditions and microbiological safety levels), licensees of food premises would be advised to investigate and find out the causes and to adopt measures to improve the hygienic conditions. Taking of follow-up samples to verify the improvement may be required.

In case of food samples containing unacceptable levels of pathogens (potentially hazardous to consumers), apart from giving advice to the licensees of food premises, actions including issuing warning letters and other enforcement actions will be considered.

### **7. What are the main changes in the revised Guidelines (May 2007 edition)?**

The main revision is in the microbiological limits for *Listeria monocytogenes*. Under the revised Guidelines, *Listeria monocytogenes* should not be detected in 25g of food under refrigeration (excluding frozen food) and food intended for infants. For other ready-to-eat food, it will be classified as unacceptable if equal to or exceeding 100 cfu/g of *Listeria monocytogenes* are found. In the past, zero tolerance policy for *Listeria monocytogenes* was adopted for all ready-to-eat food.

### **8. What are the rationales for the change of *Listeria monocytogenes* limit in the Guidelines?**

Some studies showed that most cases of listeriosis resulted from consumption of high numbers of the pathogen and are associated with the consumption of foods that do not meet standards for *Listeria*

*monocytogenes* in food, whether that standard is zero tolerance or 100 cfu/ g. On the other hand, certain foods are of relatively high risk and certain populations are more susceptible to the infection. As such, more stringent control applies to certain food items. Such approach is also adopted by other developed countries.

#### Food under refrigeration

Unlike other foodborne pathogens, *Listeria monocytogenes* is able to grow under refrigerated temperature. Hence, its number may increase under refrigerated storage. Higher level of contamination and longer duration of storage contribute to higher risk. Therefore foods kept under refrigeration at the point of sale are subject to a more stringent *Listeria monocytogenes* limit i.e. not detected in 25g.

#### Frozen food

*Listeria monocytogenes* is able to grow between 0°C and 45°C conditions; however, its growth is prohibited at temperatures below 0°C. Therefore frozen foods fall into “other ready-to-eat food” category, having a less than 100 cfu/g *Listeria monocytogenes* limit.

#### Food intended for infants

Infants are more susceptible of *Listeria monocytogenes* infection. As such, it is subject to a more stringent control and a “not detected in 25g” limit is set for food intended for infants (i.e. with age from 0 to 12 months old).

### **9. What are the high risk food items for *Listeria monocytogenes*?**

A wide range of food may be contaminated by *Listeria monocytogenes*. According to recent international epidemiological data, ready-to-eat food having a long refrigerated shelf life such as soft and semi soft cheeses, pate, cooked cold deli meat, cold-smoked fish/ seafood are relatively high risk items. However, regardless of the potential risk of the food, the industry should adhere to good manufacturing practice during food production to prevent the contamination of food with *Listeria monocytogenes*.

### **10. The revised Guidelines have included guidance notes on sampling plan for microbiological analysis. What are their purposes and factors to be considered during their application?**

The food trade may adopt a suitable sampling plan as shown in the ‘Guidance notes on sampling plan for microbiological analysis’ to monitor the safety and quality of their food products.

Application of sampling plan is an effective way to monitor the microbiological quality of food products and the effectiveness of cleansing and disinfection procedures applied in food production plants. Before choosing a sampling plan, the trade should consider the following elements –

- Purpose of inspection
- Nature of product
- Nature of the sampling and analytical procedure
- Microbiological limits
- Resources availability

For details of the principle and application of the International Committee of Microbiological Specification for Foods’ (ICMSF) sampling plan, please refer to the ICMSF publication - Microorganisms in Foods 2, Sampling for microbiological analysis: Principles and specific applications (2nd edition; 1986).

### **11. When the revised Guidelines become effective?**

The effective date is 28 May 2007.

## **Supplementary Information to Microbiological Guidelines for Ready-to-eat Food**

### **1. Why was the Supplementary Information to the Microbiological Guidelines for Ready-to-eat Food (the Supplementary Information) developed?**

To facilitate regulation, monitoring and control of microbiological quality of bottled water, edible ice and non-bottled drinks, an Ad Hoc Working Group on Microbiological Safety of Food was formed in 2008 under the Expert Committee on Food Safety to establish the microbiological criteria for bottled water and edible ice, and to review the microbiological criteria for non-bottled drinks. The criteria adopted by the Expert Committee have been described in the Supplementary Information.

## 2. What are the new microbiological criteria listed in the Supplementary Information?

The new microbiological criteria are for bottled water, edible ice and non-bottled drinks.

Types of food		Microbiological criteria		
Bottled water	Natural Mineral Waters	<u>First Examination</u>		
		<i>E. coli</i> or thermotolerant coliforms (1 x 250 ml)	Not detected in any sample	
		Total coliform bacteria (1x 250 ml)	If $\geq 1$ cfu or $\leq 2$ cfu -> a	
		Faecal streptococci (1 x 250 ml)	second examination is	
		<i>Pseudomonas aeruginosa</i> (1x 250 ml)	carried out;	
		Sulphite-reducing anaerobes (1 x 50 ml)	If $> 2$ cfu -> rejected	
		<u>Second Examination (same test volumes as for the first examination)</u>		
		Total coliform bacteria	n=4, c=1*, m=0, M=2	
		Faecal streptococci	n=4, c=1*, m=0, M=2	
		<i>Pseudomonas aeruginosa</i>	n=4, c=1*, m=0, M=2	
		Sulphite-reducing anaerobes	n=4, c=1*, m=0, M=2	
		* Results of 1st and 2nd examination		
		Bottled/Packaged Drinking Waters (Other than Natural Mineral Waters)	<i>E. coli</i>	0 /100 ml
			Total coliform bacteria	0 /100 ml
<i>Pseudomonas aeruginosa</i>	0 /250 ml			
Edible ice	Ice from ice manufacturing plants and retail outlets (packaged ice)	Total coliform bacteria	0 /100 ml	
		<i>E. coli</i>	0 /100 ml	
		Aerobic colony count	<500 /ml	
	Ice from retail business (loose ice)	Total coliform bacteria	<100 /100 ml	
		<i>E. coli</i>	0 /100 ml	
		Aerobic colony count	<1 000 /ml	
Non-bottled drinks	<i>Clostridium perfringens</i>	<100 /ml		
	<i>E. coli</i>	<100 /ml		
	<i>Salmonella</i> spp.	0 /25 ml		
	<i>Staphylococcus aureus</i>	<100 /ml		
	Others pathogens	In accordance with the Guidelines		

### 3. What is natural mineral water?

As stated in the Codex Standard for Natural Mineral Waters (CODEX STAN 108), natural mineral water is a water clearly distinguishable from ordinary drinking water. In brief, natural mineral water is originated from a protected underground source and microbiologically wholesome. The water is characterised by its content of certain mineral salt, trace elements or other constituents. It has constant composition and stable in its discharge as well as its temperature, due account being taken of the cycles of minor natural fluctuations. In addition, the water is collected under conditions 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.

### 4. Why are the faecal streptococci and sulphite reducing anaerobes tested for natural mineral waters?

These two criteria are for ensuring the hygiene of natural mineral waters for which treatment processes allowed are limited. Faecal streptococci are used as an additional indicator of human and animal faecal contamination and are more persistent than *E. coli* and coliform. These bacteria rarely multiply in water. They can be used as an indicator of treatment efficiency and for detecting water pollution, e.g. pollution of groundwater. Sulphite-reducing anaerobes, of which *Clostridium perfringens* is the most characteristic, is normally present in faeces. Clostridial spores can survive in water much longer than organisms of the coliform group and resist disinfection. Because of their longevity, they are best regarded as indicating intermittent or remote contamination. Presence of the spores should lead to investigation of filtration performance.

### 5. What is the use of the 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. In the case of assessing microbiological quality of natural mineral water, the lot should be rejected when *E. coli* is found in 250 ml in any sample. For the other microbiological criteria (total coliform bacteria, faecal streptococci,

*Pseudomonas aeruginosa* and sulphite-reducing anaerobes), the microbiological limits, i.e. 'm' and 'M', and the number of samples to be tested (n) are included. The lot should be rejected when the levels of the test microorganisms in the test volumes in any sample are found to exceed M; the lot is accepted when the levels of all the test microorganisms in the test volume in all samples are found to be less than m. The lot is also accepted, when only 1 among 5 samples (results of the first and second examination) is detected with the test microorganisms in the test volumes at a level above m and less than or equal to M. For ready-to-eat food, a "Guidance notes on sampling plan for microbiological analysis" has been prepared to provide information to the food trade on application of sampling plan in order to enhance food safety and improve food quality. This guidance is attached in Annex II of the Guidelines.

**6. What is *Pseudomonas aeruginosa* and what is its significance for microbiological quality of bottled water?**

*Pseudomonas aeruginosa* is a microorganism commonly found in the environment e.g. in soil, water, sewage, as well as faeces. It rarely causes disease in healthy people, but can infect people with weakened immunity, especially in the hospital environment. Ingestion of drinking-water contaminated with the bacterium is not an important source of infection. However, the presence of this bacterium in bottled water with subsequent growth may lead to deterioration in quality in terms of taste, odour and turbidity.

**7. Why are the microbiological criteria for packaged ice and those for loose ice different?**

The Supplementary Information has laid down more stringent requirements for packaged ice than loose ice in terms of total coliform bacteria and aerobic colony count. One of the reasons is that packaged ice is less likely to expose to environmental contamination in an intact package, while for loose ice which may have undergone some handling processes, may expose to airborne and environmental contamination. In the Centre for Food Safety 2005 survey assessing the microbiological quality of edible ice, all samples of edible ice from manufacturing plants were satisfactory for the current criteria for packaged ice.

**8. What should be done to ensure the microbiological quality of edible ice?**

The "[Guidelines on Hygienic Production and Handling of Ice in Food Premises](#)" can be found on the Centre for Food Safety website. The guidelines include hygienic practices for edible ice made by machines on premises (generally as ice cubes or ice flakes) or supplied by a specialist supplier (as packaged ice).

**9. What are non-bottled drinks?**

Generally speaking, non-bottled drinks are those drinks prepared for immediate consumption and do not require storage in sealed bottles, cans or other containers, for example, fresh fruit juice, diluted drinks prepared from concentrated fruit juice or syrup, soya bean juice, etc. Drinks sold from a manual dispensing machine also belong to this category.

**10. Why is there a licensing condition on bacteriological standards for non-bottled drinks imposed on restricted food permits and food business licences with the permission to sell non-bottled drinks?**

"Non-bottled drinks" is a restricted food specified in Schedule 2 of 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 of 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.

**11. What are the differences between the old and new microbiological criteria for non-bottled drinks?**

The old and new microbiological criteria for non-bottled drinks are compared in the table below: –

	Previous version	New version

The licensee / permittee shall take all necessary steps to ensure that the non-bottled drinks are free from contamination and that any bacteriological sample taken thereof is satisfactory. Three consecutive unsatisfactory bacteriological samples within 6 months will result in suspension of the permit / permission and additional sub-standard samples within 12 months may lead to further suspension or even cancellation of the permit / permission by the Director of Food and Environmental Hygiene. In this context, a satisfactory bacteriological sample means the following standards:-

- (a) *Clostridium perfringens* : less than 20 per ml
- (b) *Escherichia coli* : absent in 1 ml
- (c) *Salmonella* group of organisms : absent in 25 ml
- (d) *Shigella* group of organisms : absent in 25 ml
- (e) *Staphylococcus aureus* : less than 20 per ml

The licensee / permittee shall take all necessary steps to ensure that the non-bottled drinks are free from contamination and that any bacteriological sample taken thereof is satisfactory. Three consecutive unsatisfactory bacteriological samples within 6 months will result in suspension of the permit / permission and additional sub-standard samples within 12 months may lead to further suspension or even cancellation of the permit / permission by the Director of Food and Environmental Hygiene. In this context, a satisfactory bacteriological sample means the following standards:-

- (a) *Clostridium perfringens* : less than 100 cfu\* per ml
- (b) *Escherichia coli* : less than 100 cfu\* per ml
- (c) *Salmonella* species : absent in 25 ml
- (d) *Staphylococcus aureus* : less than 100 cfu\* per ml

Note : \*Colony-forming unit

## 12. When does the Supplementary Information to the Guidelines become effective?

The effective dates for the microbiological criteria are as follows: –

Microbiological criteria	Effective dates
Bottled water	1 June 2009 , except for microbiological criterion <i>Pseudomonas aeruginosa</i> which will be implemented by 2009 when testing capacity for this criterion is ready. For the two additional criteria, i.e. faecal streptococci and sulphite-reducing anaerobes, and the sampling plan for natural mineral waters, they will be implemented once additional resources are made available.
Edible ice	1 June 2009
Non-bottled drinks	The new version of the licensing condition will be imposed on newly issued restricted food permit and food business licence with the permission to sell non-bottled drinks with effect from 1 June 2009 . For existing restricted food permits and food business licences, the revised condition will take effect from 1 September 2010 .