Guidelines for the microbiological examination of ready - to - eat foods

(December 2001)

Introduction

Samples collected for surveillance and monitoring purposes are often multi-component products for which there are no microbiological standards or guidelines. Interpreting the significance of the types and levels of microorganisms reported when these foods are tested may therefore be difficult. The purpose of this document is to provide assistance in the interpretation of microbiological analyses of foods where no other microbiological criteria exist.

These guidelines identify four categories of microbiological quality for ready-to-eat foods ranging from satisfactory to potentially hazardous. This reflects both the high level of microbiological quality that is achievable for ready-to-eat foods in Australia and New Zealand and indicates the level of contamination that is considered to present a significant risk to public health. Follow-up actions appropriate to each category of microbiological quality are also recommended.

The guidelines for the microbiological examination of ready-to-eat foods apply to foods sampled at the point of sale or distribution[*] to consumers.

Ready-to-eat foods
Ready-to-eat food is food that is ordinarily consumed in the same state as that in which it is sold or distributed and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer.

Sampling

These guidelines provide quantitative levels of microbiological quality but do not provide sampling plans for the acceptance/rejection of sample lots.

Food samples may be submitted for laboratory analysis for a number of reasons and may be limited in size and number. While these guidelines will allow an assessment of the microbiological quality of a single sample of ready-to-eat food to be made, the results may not be representative of the lot from which it is derived, unless the sample has been individually prepared. Further samples may be required if an assessment as to the microbiological status of the lot is needed.

The statistical validity of a microbiological examination increases with the number of field samples analysed. For regulatory purposes, a minimum of 5 sample units from a lot is generally specified for examination. The size of the samples taken should also be adequate to

[*] Ready-to-eat foods for distribution to consumers would include those provided by airlines, hospitals and other institutions.

enable appropriate microbiological analyses to be undertaken. A minimum sample size of 100g or ml is commonly required.

The ICMSF[1] publication *Microorganisms in Foods 2, Sampling for Microbiological Analysis: Principles and Specific Applications* (1986) provides detailed information on using appropriate sampling plans.

lot A lot is defined as a quantity of food or food units produced and handled under uniform conditions. This may be restricted to a food item produced from a particular production line or piece of equipment within a certain time period (not exceeding 24 hours).

Food examination

The microbiological testing of ready-to-eat foods should be appropriate to the type of food sample being examined and to the processing it has received. Not all the organisms listed in Table 1 are equally applicable to all food groups, nor should all the organisms listed be tested for routinely. Interpretation of results should also be based on knowledge of the product components and the production process. The significance of the microbiological tests that may be conducted is discussed below.

Standard Plate Count

The standard plate count (SPC), also referred to as the aerobic plate count or the total viable count, is one of the most common tests applied to indicate the microbiological quality of food. The significance of SPCs, however, varies markedly according to the type of food product and the processing it has received. When SPC testing is applied on a regular basis it can be a useful means of observing trends by comparing SPC results over time.

Three levels of SPC are listed in Table 1 based on food type and the processing/handling the food has undergone.

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

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

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

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

[1] ICMSF International Commission on Microbiological Specifications for Foods

Indicators

Enterobacteriaceae

The family *Enterobacteriaceae* includes many bacteria that are found in the human or animal intestinal tract, including human pathogens such as *Salmonella* and *Shigella*.
Enterobacteriaceae are useful indicators of hygiene and of post-processing contamination of heat processed foods. Their presence in high numbers ($>10^4$ per gram) in ready-to-eat foods indicates that an unacceptable level of contamination has occurred or there has been underprocessing (e.g. inadequate cooking)
Testing for *Enterobacteriaceae* is not applicable to fresh fruits and vegetables or foods containing these.

Escherichia coli

The presence of *E. coli* in ready-to-eat foods is undesirable because it indicates poor hygienic conditions which have lead to contamination or inadequate heat treatment. Ideally *E. coli* should not be detected and as such a level of <3 per gram (the limit of the Most Probable Number test) has been given as the satisfactory criteria for this organism. Levels exceeding 100 per gram are unacceptable and indicate a level of contamination which may have introduced pathogens or that pathogens, if present in the food prior to processing, may have survived.

Pathogens

Coagulase-positive staphylococci

Contamination of ready-to-eat foods with coagulase-positive staphylococci is largely as a result of human contact. Contamination should be minimised through good food handling practices and growth of the organism prevented through adequate temperature controls. Unsatisfactory levels of coagulase-positive staphylococci indicate that time/temperature abuse of a food is likely to have occurred following improper handling during food preparation. A test for enterotoxin, SET, may be appropriate where levels of coagulase-positive staphylococci exceed 10^3 cfu per gram or where poor handling practices are suspected but it is likely that viable organisms may no longer be present in significant numbers. Levels of $\geq 10^4$ cfu are considered as potentially hazardous as foods with this level of contamination may result in food borne illness if consumed.

Clostridium perfringens

Unsatisfactory levels of *C. perfringens* generally occur as a result of temperature abuse where cooked foods are held at warm temperatures (<60 ºC, particularly room temperature) for extended periods of time or cooled (to 5 ºC or below) too slowly[*]. Foods associated with foodborne illness caused by *C. perfringens* include joints of meat (especially large and rolled joints) and meat and vegetable dishes such as stews and pies. The detection of high levels ($>10^3$ cfu per gram) of *C. perfringens* should result in an investigation of the food handling controls used by the food business. Levels of $\geq 10^4$ cfu per gram are considered as potentially hazardous as consumption of foods with this level of contamination may result in food borne illness.

Bacillus cereus and other Bacillus spp

An unsatisfactory level of *B. cereus* in cooked foods generally occurs as a result of inadequate temperature control. As for *C. perfringens*, cooked foods should be held at or

[*] Food Safety Standard 3.2.2 of the Australia New Zealand Food Standards Code specifies food handling controls which include temperature control requirements for food receipt, storage, processing and display.

above 60ºC or at or below 5ºC to prevent growth, or held outside this temperature range for a limited time. Foods associated with *B. cereus* food poisoning include cooked rice dishes, other cereal based foods such as pasta/noodles, dairy based deserts and meat or vegetable dishes incorporating spices. The detection of high levels ($>10^3$ cfu per gram) of *B. cereus* should result in an investigation of the food handling controls used by the food business. Levels of $\geq 10^4$ cfu pergram are considered potentially hazardous as consumption foods with this level of contamination may result in food borne illness. Other *Bacillus* species, such as *B. subtilis* and *B. licheniformis*, have also been associated with food borne illness and may also be tested for.

Vibrio parahaemolyticus

Testing for *V. parahaemolyticus* is relevant to seafoods only. High levels of *V. parahaemolyticus* ($>10^2$ per gram) in cooked seafoods indicates that the food has been inadequately cooked or cross-contaminated after cooking with subsequent time/temperature abuse and should result in an investigation of the food handling controls used by the food business. Higher levels (up to 10^2 per gram) of *V. parahaemolyticus* in raw seafoods may be expected because of natural contamination from the aquatic environment, however levels from 10^3 to 10^4 per gram in raw seafoods would indicate inadequate temperature controls since harvesting and should be considered as unsatisfactory. The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains. Levels of *V. parahaemolyticus* of $\geq 10^4$ cfu pergram are considered potentially hazardous as consumption of the food may result in food borne illness (relates to Kanagawa-positive strains).

Campylobacter

Campylobacter should not be present in ready-to-eat foods as consumption of food containing this pathogen may result in food borne illness. The detection of *Campylobacter* indicates poor food handling controls, particularly cross contamination (especially where raw poultry is handled) or inadequate cooking (e.g. raw or undercooked meat and poultry). The use of raw milk or of contaminated water may be alternative sources of *Campylobacter* that should be considered.

Salmonella

Ready-to-eat foods should be free of Salmonella as consumption of food containing this pathogen may result in food borne illness. The presence of this organism indicates poor food preparation and handling practices such as inadequate cooking or cross contamination. Consideration may also be given to investigating the health status of food handlers on the premises who may have been suffering from salmonellosis or asymptomatic carriers of the organism.

Listeria monocytogenes

Listeria monocytogenes is widespread in the environment and can be isolated from a wide variety of foods. Its detection in ready-to-eat foods which have not undergone a listericidal treatment, therefore, does not immediately indicate a problem with food practices within the food establishment. Higher levels of *L. monocytogenes* (10^2 cfu per gram), however, do indicate a failure with food handling controls and based on current epidemiological evidence are considered a public health risk. Foods in which all components have been cooked in the final food preparation, or have received some other listericidal treatment, should be Listeria free. The detection of *L. monocytogenes* in such foods indicates the food was inadequately cooked or the food was contaminated post preparation. Additionally, the detection of *L. monocytogenes* in foods which have been prepared specifically for “at risk” population

groups such as the elderly, immunocompromised and infants should be considered as potentially hazardous.

Categories of microbiological quality

Four categories of microbiological quality have been assigned based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous.

Satisfactory – results indicate good microbiological quality.
No action required.

Marginal – results are borderline in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food.
Action: Re-sampling may be appropriate. Premises that regularly yield borderline results should have their food handling controls investigated.

Unsatisfactory – results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices.
Action: Further sampling, including the sampling of other foods from the food premise may be required and an investigation undertaken to determine whether food handling controls and hygiene practices are adequate.

Potentially Hazardous – the levels in this range may cause food borne illness and immediate remedial action should be initiated.
Action: Consideration should be given to the withdrawal of any of the food still available for sale or distribution and, if applicable, recall action may be indicated. An investigation of food production or handling practices should be instigated to determine the source/cause of the problem so that remedial actions can commence.

Table 1. Guideline levels for determining the microbiological quality of ready-to-eat foods

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Standard Plate Count				
Level 1.	$<10^4$	$<10^5$	$\geq 10^5$	
Level 2.	$<10^6$	$<10^7$	$\geq 10^7$	
Level 3.	N/A	N/A	N/A	
Indicators				
*Enterobacteriaceae**	$<10^2$	10^2-10^4	$\geq 10^4$	
Escherichia coli	<3	3-100	≥100	**
Pathogens				
Coagulase +ve staphylococci	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$ SET +ve
Clostridium perfringens	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
Bacillus cereus and other pathogenic *Bacillus* spp	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
Vibrio parahaemolyticus #	<3	<3 -10^2	10^2 –10^4	$\geq 10^4$
Campylobacter spp	not detected in 25g			detected
Salmonella spp	not detected in 25g			detected
Listeria monocytogenes	not detected in 25g	detected but $<10^2$ ‡		$\geq 10^2$ ##

* *Enterobacteriaceae* testing is not applicable to fresh fruits and vegetables or foods containing these.

**Pathogenic strains of *E. coli* should be absent.

V. parahaemolyticus should not be present in seafoods that have been cooked. For ready-to-eat seafoods that are raw, a higher satisfactory level may be applied ($<10^2$ cfu/g)
The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains.

‡ Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.

The detection of *L. monocytogenes* in ready-to-eat foods prepared specifically for "at risk" population groups (the elderly, immunocompromised and infants) should also be considered as potentially hazardous.

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