



# Microbial detection and safety methods

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# Microbial Detection Methods

## Learning objectives:

Know the aims of microbiological examinations of foods, and the need to use standardized methods

Understand the need for sampling plans and how they are used to assess product safety

Be able to describe the principles and give examples of common methods of detecting microbes (or their products) in foods, including:

- a. Conventional microbiological methods (cultivation)
- b. Immunological methods (ELISA) & enzyme assays (bioluminescence)
- c. Molecular detection methods (PCR, qPCR)

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# Reasons for microbiological examination of food

1. meet microbiological specifications and standards
2. assess safety for consumers
3. help determine shelf life / stability
4. know level of sanitation during processing/handling
5. for raw ingredients – to predict if heating/preservation steps will be sufficient to meet specifications
6. determine sources of post-heating contaminants

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# Standards, specifications and guidelines

## Microbiological standard

Legal requirement; enforced by appropriate regulatory agency; mandatory. Failure to comply has a range of penalties.

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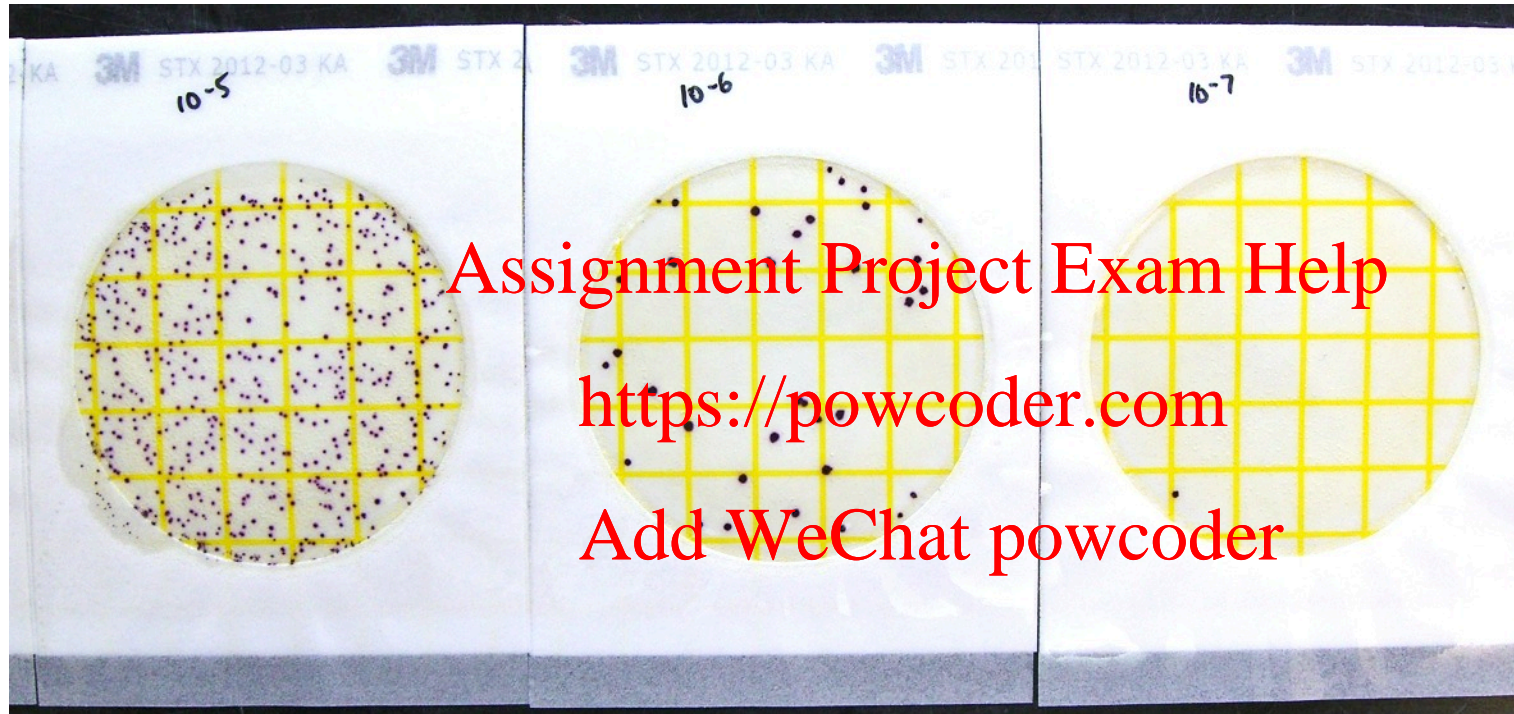
In Australia there are local, state and national regulations

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Food Standards Code where it states, "*The Code is enforced by state and territory and New Zealand agencies and the Department of Agriculture for imported food*"

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# Microbiological standard



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Example: If the microbiological standard required that there must be less than  $10^6$  CFU/ml of *S. aureus* in the product, and you get the above results back from the lab, what do you do with that batch of food? (assume 1 ml sample of each dilution added to each Petrifilm plate)



# Standards, specifications and guidelines

## Microbiological specification

As applied in commerce; failure to meet this results in rejection, e.g. the buyer refuses the food sent to them

## Microbiological guideline

Advisory only; Used to monitor acceptability of production process

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Microbiological standard

Legal requirement

Microbiological specification

Applied in commerce

Microbiological guideline

Advisory only

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**FSANZ**

Go to the Food Standards Australia and New Zealand website

<https://www.foodstandards.gov.au>

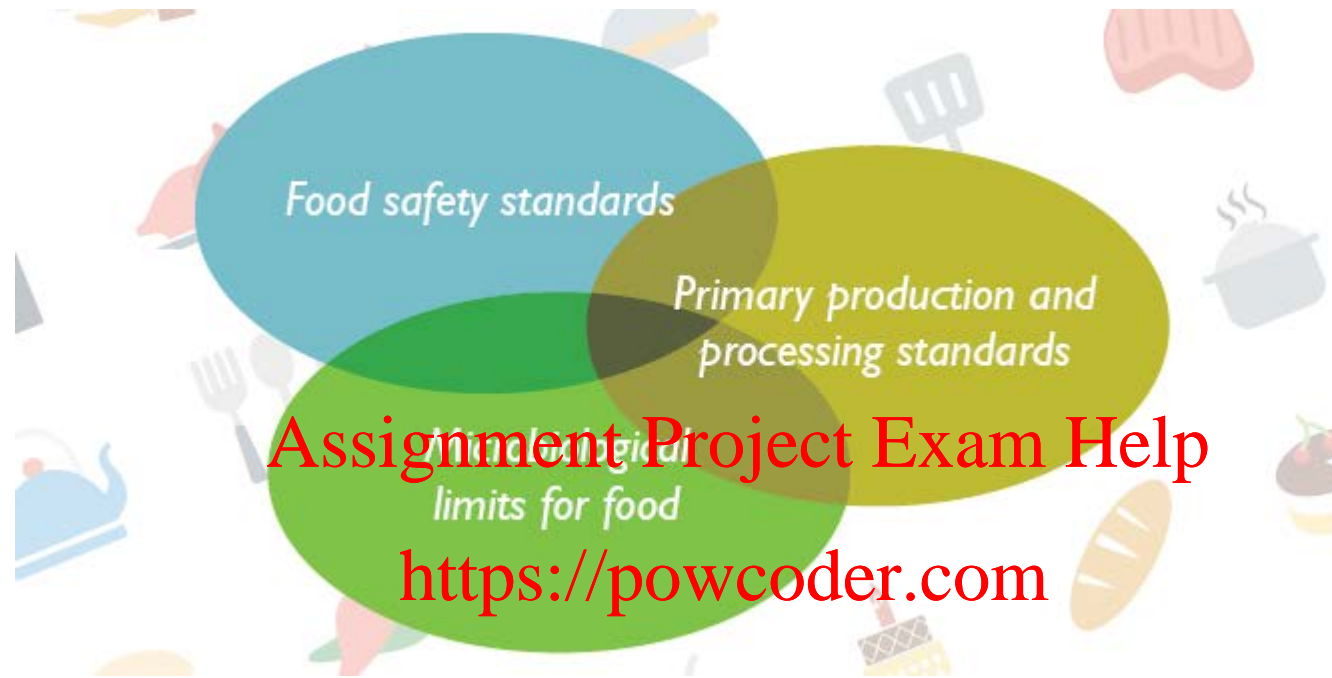
Explore

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Food Standards Code <https://powcoder.com> > Food safety standards

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**This section outlines the regulatory requirements related to food safety and links to a series of useful guides and fact sheets on specific food safety topics.**

Food safety is covered by several standards in the Australia New Zealand Food Standards Code. These standards aim to lower the incidence of foodborne illness by strengthening food safety and traceability throughout the food supply chain, from paddock to plate.

The standards are:

- Food Safety Standards (Chapter 3 – Australia only)
- Primary Production and Processing Standards (Chapter 4 – Australia only)
- Microbiological Limits for Food (Standard 1.6.1)



# Food Safety Standards

These standards apply to all food businesses in Australia only. Food businesses in New Zealand are required to comply with New Zealand's *Food Act 1981* and the regulations and standards under this Act. For more information visit the [Ministry for Primary Industries website](#).

The food safety standards aim to lower the incidence of foodborne illness. They place obligations on Australian food businesses to produce food that is safe and suitable to eat, and also place health and hygiene obligations on food handlers.

A food business is any business or activity that involves the handling of any type of food for sale, or the sale of food in Australia.

Charities, community groups and businesses operating from a private home or at temporary events are exempt from some of the requirements in the food safety standards. These groups and businesses can contact their [local enforcement authority](#) for further information.

## Food Safety Standards

There are five food safety standards:

**3.1.1 Interpretation and application**

**3.2.1 Food safety programs**

**3.2.2 Food safety practices and general requirements**

**3.2.3 Food premises and equipment**

**3.3.1 Food safety programs for food service to vulnerable persons**

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### 3.1.1 Interpretation and application

This standard defines the terms used in the food safety standards, specifies these standards apply to Australia only and that food businesses and food handlers must comply with their respective requirements.

The guide for this standard is *Safe Food Australia*.

### 3.2.1 Food safety programs

This standard enables states and territories to require food businesses to implement a food safety program and sets out the general requirements for these programs.

A food safety program identifies the food safety hazards associated with a business's food handling activities and indicates how the business will monitor and control these hazards.

For more information, see the Food Safety Programs web page.

### 3.2.2 Food safety practices and general requirements

This standard specifies food handling controls related to the receipt, storage, processing, display, packaging, transportation, disposal and recall of food. Other requirements relate to the skills and knowledge of food handlers and their supervisors, the health and hygiene of food handlers, and the cleaning, sanitising and maintenance of food premises and equipment.

Some exemptions apply to charities and community groups and to businesses operating from a private home or temporary premises (the local enforcement authority should be contacted for advice).

The guide for this standard is *Safe Food Australia*. Fact sheets and more information on these requirements are available



### 3.2.3 Food premises and equipment

This standard sets out Australian requirements for the design and construction of food premises, fixtures, fittings, equipment and food transport vehicles. It aims to ensure that, where possible, the layout of the premises minimises opportunities for food contamination. These requirements should help food businesses meet the food safety requirements of Standard 3.2.2.

Some exemptions apply to food businesses operating from a private home or at temporary premises — the local enforcement authority should be contacted for advice.

The guide for this standard is Safe Food Australia

View our InfoBites for key information on the food safety standards in the Code

### 3.3.1 Food safety programs for food service to vulnerable persons

This standard requires Australian food businesses that prepare food for service to vulnerable people to implement a food safety program. This includes businesses providing food to hospital patients, aged care residents and children in childcare centres, who are generally at greater risk of foodborne illness. It usually also includes delivered meal organisations.

The guide for this standard is Food Safety Programs for Food Service to Vulnerable Persons – A guide to Standard 3.3.1(pdf 1.44 Mb).

For more information, see the Food Safety Programs web page.



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**Standard 1.6.1 Microbiological limits in food** aims to prevent foodborne illness from food containing unsafe levels of microorganisms. The standard sets out how to determine whether a specific lot of food has an unacceptable level of microorganisms. Maximum permissible limits for particular microorganisms in different food groups are listed in the accompanying Schedule 27.

In addition to the standard, FSANZ has developed guideline levels for certain other microorganisms and foods. If these guideline levels are exceeded, it generally indicates a problem in the food production process or hygiene procedures that needs to be addressed.



- Compendium of Microbiological Criteria for Food
- Guidance on the Application of Microbiological Criteria for *Listeria monocytogenes* in RTE Food
- Safe Food Australia
- Codex

Microbiological testing can play an important role in food safety management providing the reason for testing is clear and the test applied is appropriate.

- Microbiological criteria support decision making about a food or process when microbiological testing is used.
- A microbiological criterion provides microbiological limits that are considered appropriate to a food at a specific point of the food chain and states the actions to be taken when the criterion is not met.
- Microbiological criteria that are applied to determine the safety of a food lot (food safety criteria) are included in the Australia New Zealand Food Standards Code (the Code).
- Other microbiological criteria (process hygiene criteria) can be developed and applied at various stages throughout the food chain to indicate whether the food safety controls in place are working as intended.

The Compendium of Microbiological Criteria for Food is a compilation of process hygiene criteria that have been established for specific food commodities and microbiological guideline criteria used for ready-to-eat foods.

The development of process hygiene criteria is an ongoing activity associated with the review of Standard 1.6.1. The Compendium will be continually updated to include new criteria as it is established.

See Readings in Canvas for Compendium of Microbiological Criteria for Food

see FSANZ for current and complete info.

The microbiological criteria used by food regulatory agencies generally include:

**food safety criteria:** microbiological criteria that are applied to determine the safety of a food lot.

**process hygiene criteria:** microbiological criteria applied to verify hygiene measures or control of process. They are applied at a specified point in the manufacturing process.

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

## Compendium of Microbiological Criteria for Food

(October 2016)



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# There are four categories of microbiological assessment defined based on the detection or level of microorganisms (pathogens or indicator organisms) found by testing

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



# Example for detecting pathogens in RTE food

Table 1. Interpreting results for testing of pathogenic microorganisms in ready-to-eat food<sup>2</sup>

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

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# Example of testing for indicator organisms in RTE food

Table 2. Interpreting results for testing of indicator organisms in ready-to-eat foods

Indicator	Result (cfu/g)	Interpretation	Likely cause	Actions
<u>Enterobacteriaceae</u> <sup>4</sup> (includes coliforms)	>10 <sup>4</sup>	Unsatisfactory	For processed foods indicates that contamination has occurred post processing (cross contamination from food contact surfaces, raw products or food handlers) or there has been inadequate processing. Poor temperature/time control may also be a contributing factor.	<ul style="list-style-type: none"><li>• Review:<ul style="list-style-type: none"><li>— processing controls used (such as cooking temperatures)</li><li>— cleaning and sanitising practices for premises and equipment</li><li>— food handler hygiene</li><li>— time and temperature control.</li></ul></li></ul> Additional food or environmental samples may be required for investigation.
	10 <sup>2</sup> – 10 <sup>4</sup>	Marginal	Some cross contamination or inadequate processing indicated.	Proactive investigation to ensure processing and hygiene controls are being implemented. Results may need to be compared with other food samples from the production environment for interpretation.
	<10 <sup>2</sup>	Satisfactory		

# Sampling of foods

- If you don't collect a representative sample then even perfect microbiological testing will not provide a true estimate

## Sampling Plans

- Define the probability of detecting a microorganisms or other hazards in a lot
- None can ensure the absence of a particular hazard
- Several means of statistical sampling of batches/lots of food product
  1. single attribute plan
  2. multiple attribute plan

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# Attribute sampling plans

**Qualitative** analytical results (presence/absence) or **quantitative** results that have been grouped (e.g.  $<10$  cfu/g, 10 to 100 cfu/g,  $>100$  cfu/g)

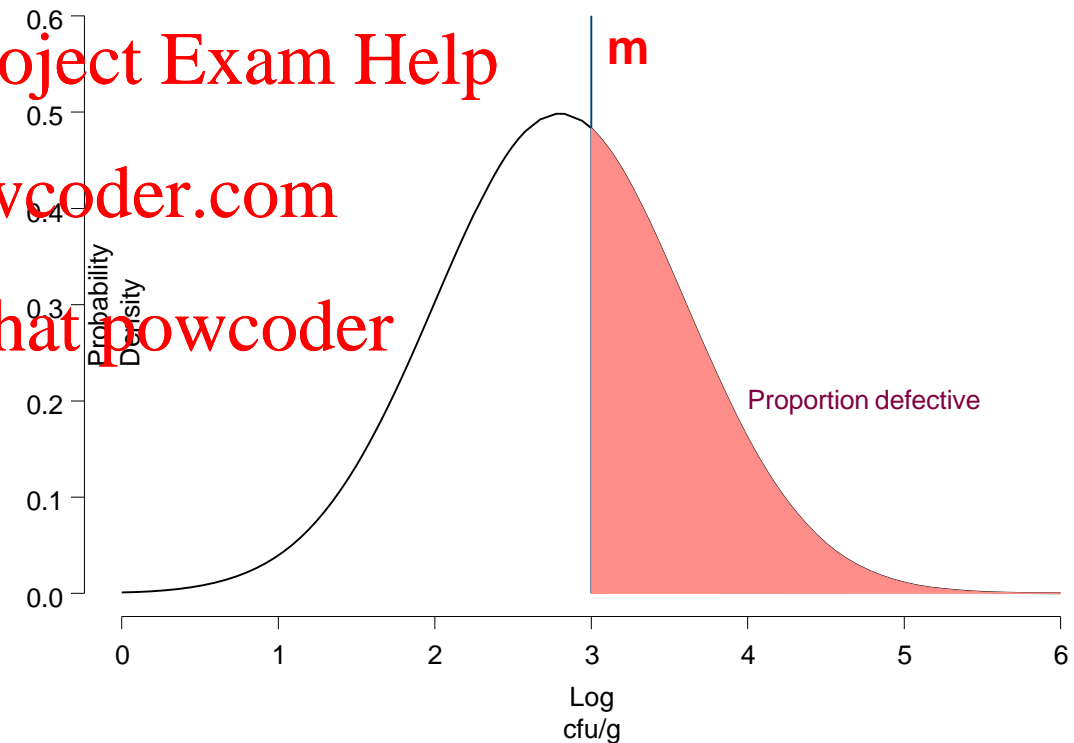
**Two-class sampling** plans designed to decide on acceptance or rejection of a lot consist of

**n** – number of sample units to be chosen independently and randomly from the lot

**m** – a microbiological limit (i.e. in cfu/g); a sample is defined to be positive, if its microbial content exceeds this limit

**c** – maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit  $m$ ; for pathogens  $c$  is usually set to 0

Two-class sampling plan:



# Attribute sampling plans

**Qualitative** analytical results (presence/absence) or **quantitative** results that have been grouped (e.g. <10 cfu/g, 10 to 100 cfu/g, >100 cfu/g)

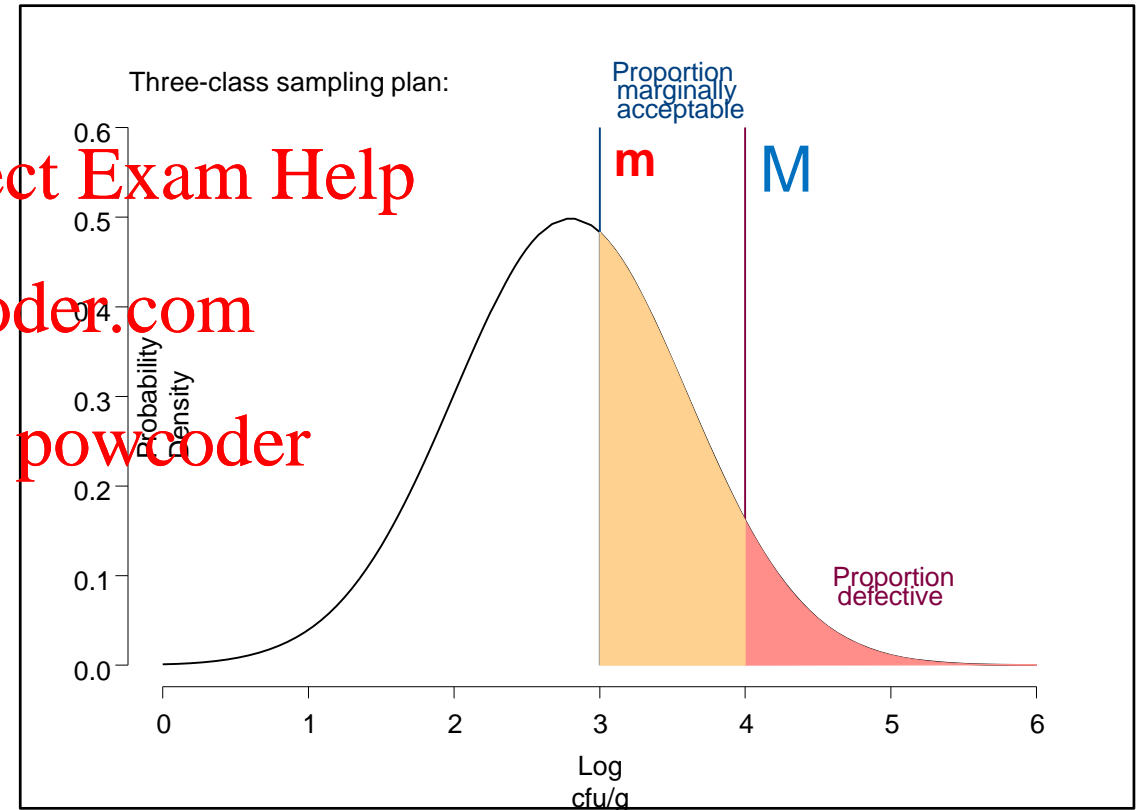
**Three-class sampling** plans designed to decide on lot acceptance based on two proportions

Marginally acceptable: between  $m$  and  $M$

Defective: above  $M$

$n$  – number of sample units to be chosen independently and randomly from the lot

$c$  – maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit  $m$ ; for pathogens  $c$  is usually set to 0





**n** – number of samples to be taken

**c** – maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit  $m$

**m** – a count which, if exceeded by any of the test samples, would lead to rejection of the batch

Column 1	Column 2 (n)	Column 3 (c)	Column 4 (m)
<b>Pepper, paprika and cinnamon</b>			
Salmonella	5	0	not detected in 25 g
<b>Dried, chipped, desiccated coconut</b>			
Salmonella	10	0	not detected in 25 g
<b>Cocoa powder</b>			
Salmonella	5	0	not detected in 25 g
<b>Cultured seeds and grains (bean sprouts, alfalfa etc)</b>			
Salmonella	5	0	not detected in 25 g
<b>Processed egg product</b>			
Salmonella	5	0	not detected in 25 g

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QUS:

- What type of sampling plan is this?
- Why do you think the microbiological limits for these foods is so strict? (consider each food here)





**n** – number of samples to be taken

**c** – maximum number of test samples which may fall into the marginally acceptable category before rejection

**m** – a count which separates good quality from marginal quality and which most test samples should not exceed

**M** – a count which, if exceeded by any of the test samples, would lead to rejection of the batch

#### Microbiological limits in food

Column 1	Column 2 (n)	Column 3 (c)	Column 4 (m)	Column 5 (M)
<b>All cheese</b>				
<i>Escherichia coli</i>	5	1	10/g	10 <sup>2</sup> /g
<b>Raw milk cheese</b>				
<i>Salmonella</i>	5	0	not detected in 25 g	
Staphylococcal enterotoxins	5	0	not detected in 25 g	
<b>Soft and semi-soft cheese (moisture content &gt; 39%) with pH &gt; 5.0</b>				
<i>Salmonella</i>	5	0	not detected in 25 g	
<b>Dried milk</b>				
<i>Salmonella</i>	5	0	not detected in 25 g	
<b>Unpasteurised milk for retail sale</b>				
<i>Campylobacter</i>	5	0	not detected in 25 mL	

#### QUS

- What type of sampling plan is applied to cheese?
- What type of tests would be required to generate these results?

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**Refer to Readings**

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