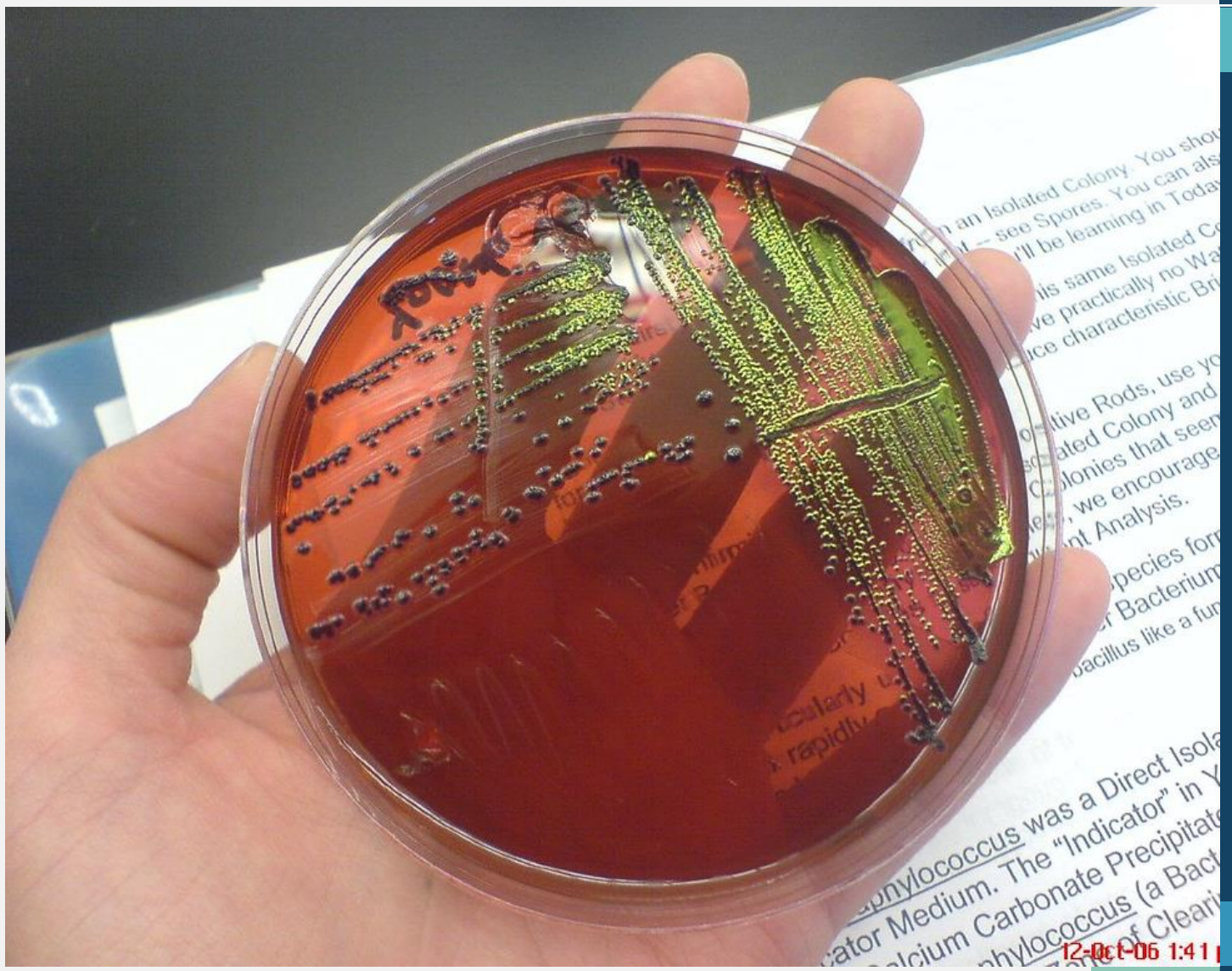

IMG 222

Microbiological criteria and sampling plan

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Content

Microbiological criteria

Sampling plan

DEFINITION

‘Microbiological criterion’ means a criterion defining the acceptability of a product, process, or food lot.

The criteria are based on:

Absence or Presence

Number of microorganisms

The quantity of their toxins/metabolites in samples

per unit(s) of mass, volume, area or lot.

Microbiological criteria give guidance on the acceptability of foodstuffs and their manufacturing processes.

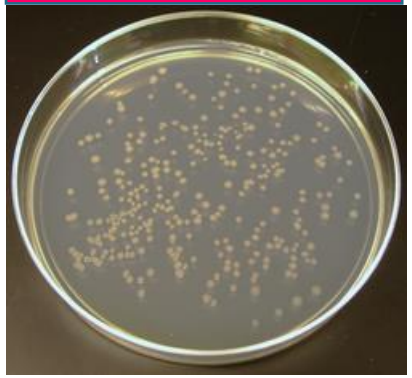
A judgment or decision can be made by the food businesses and competent authorities to manage and monitor the safety of foodstuffs based on the criteria.

5 components of microbiological criteria

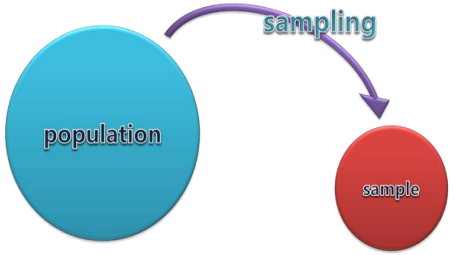
A statement of **food** to which the criterion applies.

A statement of the **organisms** of concern and/or their **toxins**

The **analytical methods** for their detection and quantitation



Number and **size** of samples taken from a batch of food or a point in a processing line



Microbiological limits appropriate for the food

Probiotic strain category	Test	Method	Acceptance criteria (CFU/g)
Contaminant microorganisms			
Non-spore-forming	Non-lactic acid bacteria	ISO 13559/ IDF 153	Not more than 5×10^3
	Total yeasts and molds	USP <2021>	Not more than 100
Spore-forming	Total yeasts and molds		Not more than 100
Yeasts and molds	Total aerobic microbial count		Not more than 1×10^3
Specified microorganisms			
Non-spore-forming, spore-forming, yeasts and molds	*Escherichia coli* *Salmonella* spp.	USP <2022>	None detected in 10 g

Microbiological criteria may be established and used by:

Food business operators

E.g. Food manufacturer used MC to validate and verify their food safety management procedures, or assessing the acceptability of their foodstuffs, handling, and distribution

National Authorities.

E.g. Ministry of Health. MC is used to set national baselines/ limits for certain microbial contaminants

Two categories of microbiological criteria:

Mandatory

A microbiological standard that should contain **limits only for pathogens** of public health significance

Food exceeded the limit may be rejected, destroyed, reprocessed etc..

Advisory

A microbiological end product specification intended to increase **assurance that hygienic significance has been met**

A microbiological guideline applied to **monitor hygiene**

Types of microbiological criteria

Microbiological standards:

A mandatory criterion that is part of a law or regulations. It is a legal requirement that foods must meet and is enforceable by the appropriate regulatory agency.

Microbiological guidelines:

Microbiological criteria which provide advice to food manufacturers about acceptable or expected microbial levels when the food production process is under control when applying best practices (e.g. GMP, HACCP).

Microbiological specifications:

Contractual agreements between a manufacturer and a buyer to check whether the food are of required quality. Failure of the supplier to meet the specification will result in rejection of the batch or a lower price

A microbiological criterion will stipulate that:

A TYPE of microorganism

e.g. *Salmonella*

A GROUP of microorganisms

e.g. *Coliforms*

TOXIN produced by a microorganism

e.g. *Aflatoxins*

MUST EITHER

Not be present at all

Be present in only a limited number of samples

Be present in less than a specified number or amount in a given quantity of a food or food ingredient.

Microbiological standards

TABLE II
MYCOLOGICAL CONTAMINANT

(1) *Food*	*(2)* *Mycological Contaminant*	*(3)* *Maximum permitted proportion in microgram per kilogram (μg/kg)*
Groundnuts for further processing	Aflatoxin	15
Milk	Aflatoxin	0.5
Others	Aflatoxin or any other mycotoxins	5

Fifteenth Schedule (Regulation 39)

MICROORGANISMS AND THEIR TOXINS

TABLE I

MICROBIOLOFICAL STANDARD

	MICROBIOLOGICAL STANDARD		
(1) ***Food***	***(2)*** ***Total Plate Count at 37°C for 48 hr.***	***(3)*** ***Coliform Count at 37°C for 48 hr.***	***(4)*** ***Escherichia coli Count***
Pasteurized milk, pasteurized cream and milk powder (including full cream and skim milk powder)	10^5 per g or per ml.	5x10 per g or per ml	
Ice cream … … …	$5x10^4$ per g	100 per g	Absent in 1 g
Meat and meat product ready for consumption, excluding meat and meat product in hermetically sealed containers.	10^6 per g	5x10 per g	
Fish and fish product ready for consumption, excluding fish and fish product in hermetically sealed containers.	10^6 per g	5x10 per g	
Infant Formula … … …	10^4 per g	10 per g	
Liquid egg, liquid egg yolk and liquid egg white.	$5x10^4$ per g	5x10 per g	
Dried liquid egg, dried liquid egg yolk and dried liquid egg white.	$5x10^4$ per g	5x10 per g	

Microbiological standard in Malaysia

Microbiological guideline

- Guideline is used when there is no established microbiological standard
- To reflect the safety and hygienic quality of the food
- Provide assistance to officers in the interpretation of microbiological analyses and give recommendation on the appropriate follow-up action to monitor & control food safety
- E.g. TPC, hygiene indicator organism, specific foodborne pathogens
- Classification: satisfactory, borderline, unsatisfactory

TPC: Quality indicator

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

Food Category [a]	Examples	Result (colony-forming unit (cfu)/g)		
		Satisfactory	Borderline	Unsatisfactory
1. Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container [b]	Canned products such as tuna, salmon, corned beef, soups, stews, desserts and fruit; ultra-high-temperature (UHT) products	<10	N/A	Note [c]
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	$<10^3$	10^3-$<10^5$	$\geq 10^5$
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	$<10^4$	10^4-$<10^7$	$\geq 10^7$
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	$<10^4$	10^4-$<10^6$	$\geq 10^6$
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.)	$<10^5$	10^5-$<10^7$	$\geq 10^7$

Coliforms, *E. coli*: Hygiene indicator

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 [a]	$<10^2$	10^2 - $\leq 10^4$	$> 10^4$
Escherichia coli [b]	<20	20 - $\leq 10^2$	$> 10^2$

* To be implemented when the testing capacity for this criterion is ready.

Source: Microbiological Guidelines for Food, Centre for Food Safety, Hong Kong

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
Campylobacter spp. (thermotolerant)	n.d. in 25g	N/A	Detected in 25g
Escherichia coli O157 (and *other Shiga toxin-producing *E. coli* (STEC))	n.d. in 25g	N/A	Detected in 25g
Salmonella spp.	n.d. in 25g	N/A	Detected in 25g
Vibrio cholerae (O1 and O139)	n.d. in 25g	N/A	Detected in 25g
Shigella spp. [a]	n.d. in 25g	N/A	Detected in 25g
Listeria monocytogenes			
● For refrigerated food [b] (excluding frozen food) or food intended for infants	n.d. in 25g [c]	N/A	Detected in 25g [c]
● For other ready-to-eat food	< 10 [d]	$10 - \leq 100$ [d]	> 100 [d]
Vibrio parahaemolyticus	< 20	$20 - \leq 10^3$	$> 10^3$
Staphylococcus aureus and other coagulase-positive staphylococci	< 20	$20 - \leq 10^4$	$> 10^4$
Clostridium perfringens	< 10	$10 - \leq 10^4$	$> 10^4$
Bacillus cereus	$< 10^3$	$10^3 - \leq 10^5$	$> 10^5$

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

* To be implemented when the testing capacity for this criterion is ready.

Source: Microbiological Guidelines for Food, Centre for Food Safety, Hong Kong

Microbiological specification

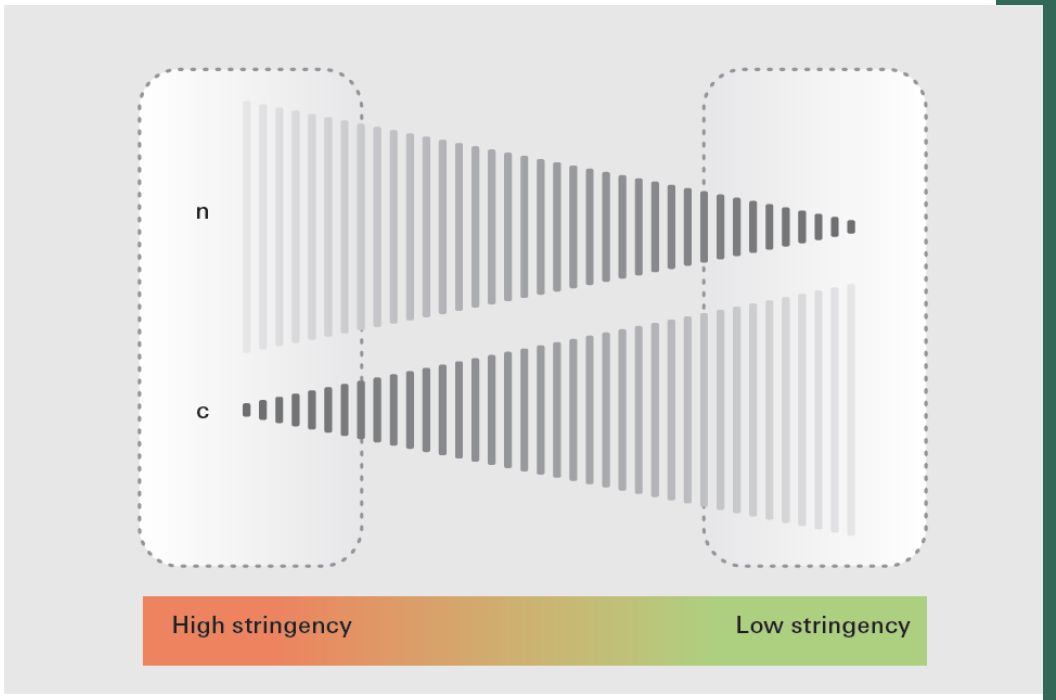
Parameter		n	c	m	M	Analytical unit
Infant Formula						
Pathogen(s)	*Salmonella*	60	0	0		25g
	C. sakazakii	30	0	0		10g
Hygiene indicators	Aerobic mesophilic counts	5	0	500		1g
	Enterobacteriaceae	10	0	0		10g
Confectionery						
Pathogen(s)	*Salmonella*	10	0	0		25g
Hygiene indicators	Aerobic mesophilic counts	5	0	10^4		1g
	Enterobacteriaceae	5	0	10		1g

An illustrative example of different stringencies applied to microbiological specifications. RM – sucrose used for infant formula (no further kill step) and used for confectionery (used as wet mix; further microbial kill step applied).

Example:

Microbiological specification of raw material (sucrose) used for infant formula and confectionary

(Source: NESTLE)



Interrelationship between n and c for microbiological specification stringency

When the microbiological criteria are needed?

- Evidence of actual or potential **hazards** to health
- **Effect of further processing** on the likely microbiological status of the food and intended use of the product
- Likelihood and consequences of **microbial contamination** and/or **growth** during subsequent handling, storage and use

SAMPLING PLAN

- A statement of the criteria of acceptance applied to a lot based on appropriate examinations of a required number of sample units by specified methods
- Consists of a sampling procedure and decision criteria and may be a two-class or a three-class plan
- Sampling plan measure compliance with performance standard. E.g. *Salmonella*

SAMPLING PLAN

Zero tolerance policies for food pathogens

- *E. coli* O157:H7 (325 grams raw product)
- Non-O157 Shiga toxin producing E. coli (325g N=60 trim sample)
- *L. monocytogenes* (25 grams RTE product, presence in food contact surface swab, 25 grams pasteurized egg product)
- *Salmonella* (325 grams RTE product or 100 grams pasteurized egg product

Assumption for sampling

- Uniform manufacturing conditions
- Equal probability of contamination throughout the lot ("homogeneous distribution")
- Independent, random sampling (equal probability of sampling throughout the lot)

International Commission on Microbiological Specifications for Foods ICMSF (1974) suggested 2 types of sampling plan:

Two class plan

Three class plan

SAMPLING PLAN

Two class plan

- Two attributes, i.e, presence or absence of an organism in a given sampling unit.
- Applied for more hazardous organisms. e.g. *Clostridium botulinum*.
- Two-class plan: n, c, m

Three class plan

- Three attributes and can divide a lot into three categories: acceptable (n,m); ▫ unacceptable (>M) and ▫ marginally acceptable(C).
- Three-class plan: n, c, m, M

n: number of sample unit from a lot

c: max acceptable number of sample that exceed microbiological criteria

m: max number of bacteria per gram

M: quantity to prepare acceptable quality vs unacceptable quality (max permitted level)

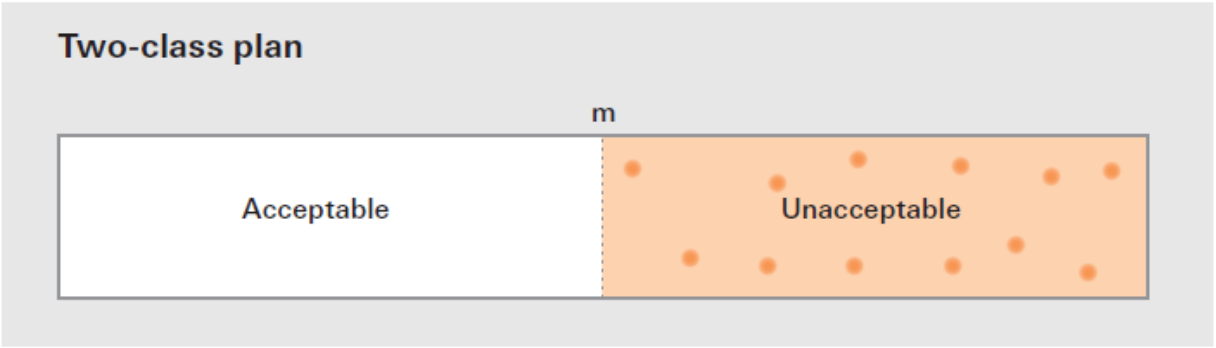
Two Class Plan

- Used to accept or reject a larger batch of food
- eg. **n=5, c=0, m=0**
- 5 individual units of the lot must be free from organism of concern)
- If any unit is positive, the entire lot will be rejected.

n: number of sample unit

c: max acceptable number of sample

m: max number of bacteria per gram



The microbiological limit (m) separates the quality of a lot between acceptable and unacceptable in a two-class plan

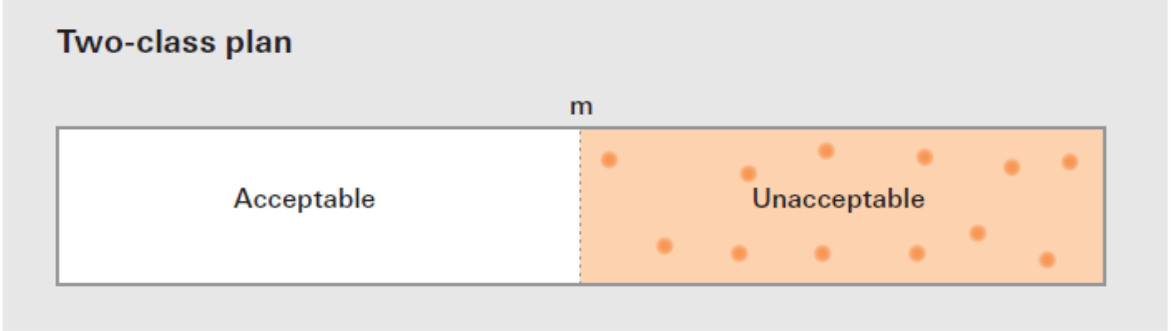
Two Class Plan

- **n=5, c=2, m=100/gram**
- If 100 coliforms/g is allowed in 2/5 units. After the five units have been examined for coliforms, the lot is acceptable if no more than 2/5 contain as many as 100 coliforms/g

n: number of sample unit

c: max acceptable number of sample

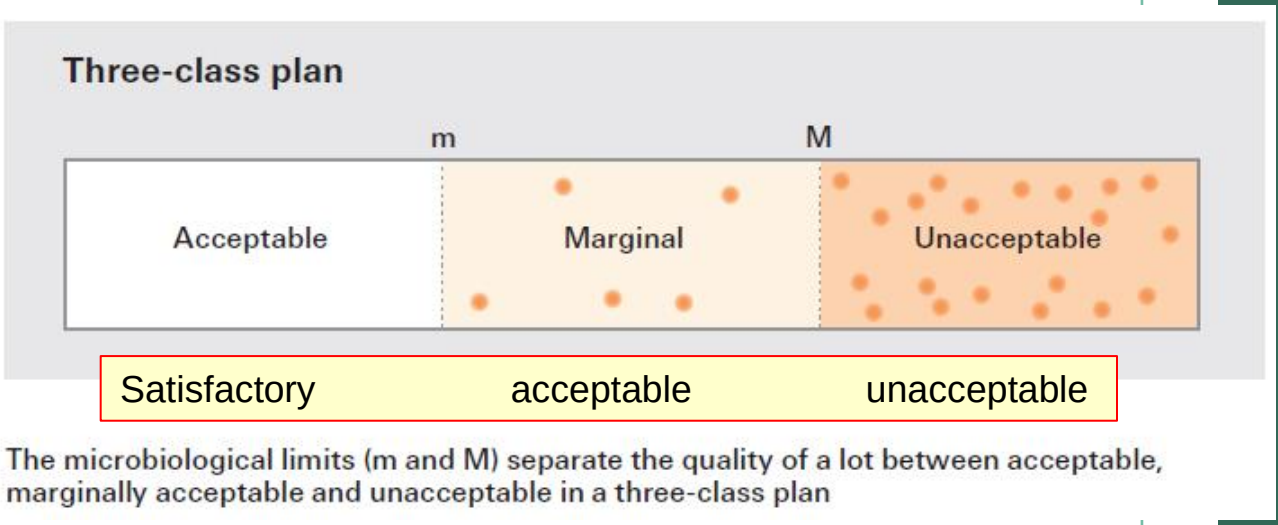
m: max number of bacteria per gram



The microbiological limit (m) separates the quality of a lot between acceptable and unacceptable in a two-class plan

Three Class Plan

- Frequently used to examine for hygience indicator
- Such plan can be used to separate the quality of a lot into acceptable, marginally acceptable, and unacceptable
- Eg: Standard plate count (SPC) shall not exceed 10^6/g (M) or be higher than 10^5/g from three or more of five units.
- **n=5, c=2, m=10^5, M=10^6.**
- If any of the 5 units exceeds 10^6/g, the entire lot is rejected.
- If less than c sample (2) units give results above m, the lot is acceptable.



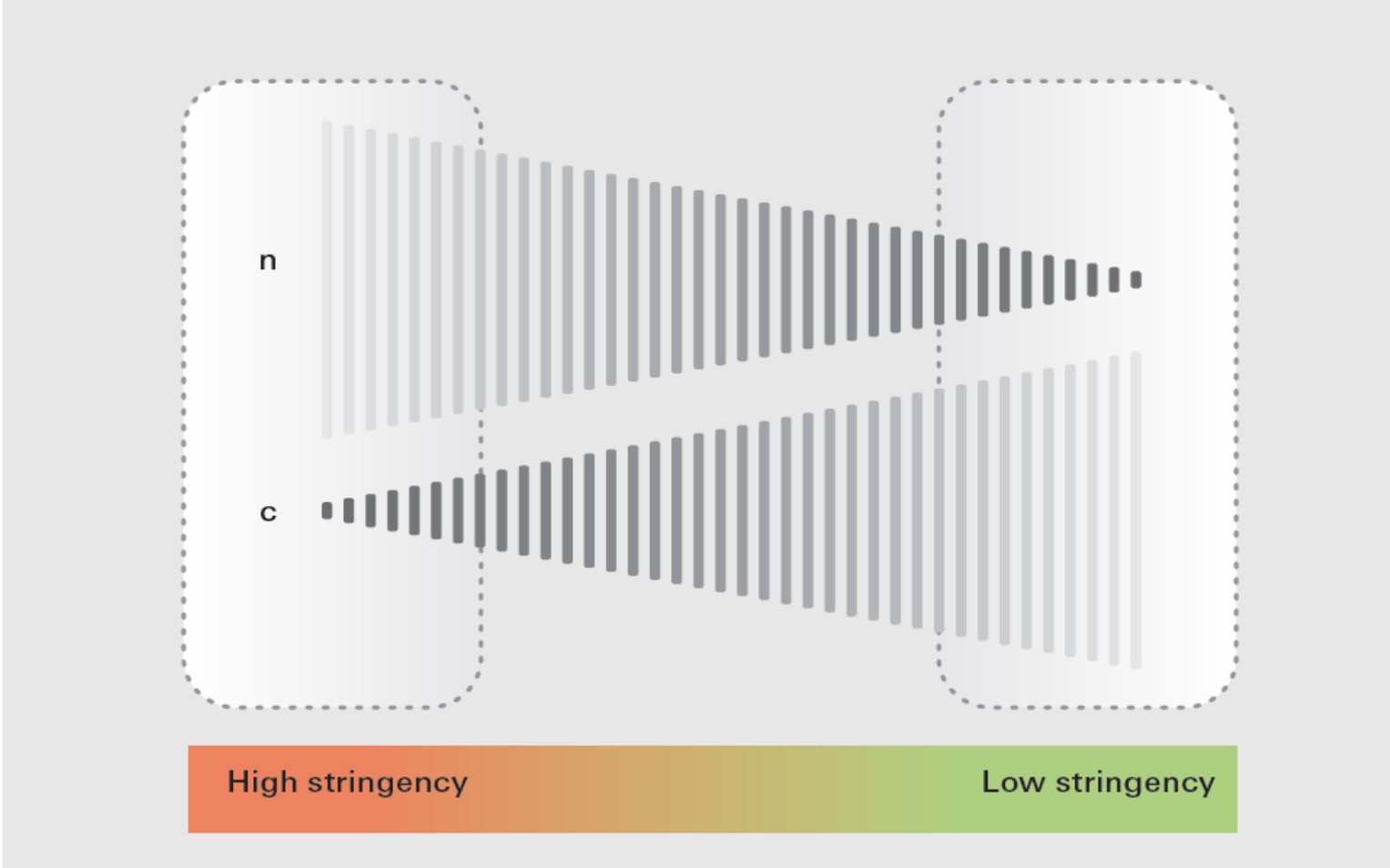
The microbiological limits (m and M) separate the quality of a lot between acceptable, marginally acceptable and unacceptable in a three-class plan

Foodborne Hazards

Three types of foodborne hazards have been recognized

1. Severe hazards: Due to potentially hazardous organisms Eg: *C. botulinum*
2. Moderate hazards:
   - organisms having extensive spread: Eg. Enteropathogenic *Escherichia coli* (EPEC)
   - organisms with limited spread: Eg. *S. aureus*
3. Low hazards:
   - organisms causing mild hazard or non-pathogenic organisms responsible for food spoilage
   - Eg. yeast and moulds.

		Conditions in which food is expected to be handled and consumed after sampling in the usual course of events		
Degree of concern relative to utility and health hazard		**Conditions reduce degree of concern**	**Conditions cause no change in concern**	**Conditions may Increase concern**
Utility: General contamination, reduced shelf life, incipient Spoilage		**Increase shelf life** **Case 1** Three class n=5, c=3	**No change** **Case 2** Three class n=5, c=2	**Reduce shelf life** **Case 3** Three class n=5, c=1
Indicator: Low, indirect hazard		**Reduce hazard** **Case 4** Three class n=5, c=3	**No change** **Case 5** Three class n=5, c=2	**Increase hazard** **Case 6** Three class n=5, c=1
Moderate hazard: Not usually life threatening, usually no sequelae, normally of short duration, symptoms self-limiting, can be severe discomfort		**Case 7** Three class n=5, c=2	**Case 8** Three class n=5, c=1	**Case 9** Three class n=10, c=1
Sample size 25g	**Serious hazard:** Incapacitating but not usually life threatening, sequelae are rare, moderate duration	**Case 10** Two class n=5, c=0	**Case 11** Two class n=10, c=0	**Case 12** Two class n=20, c=0
	Severe hazard: For the general population or in foods targeted for susceptible populations, causing life threatening or substantial chronic sequelae or illness of long duration	**Case 13** Two class n=15, c=0	**Case 14** Two class n=30, c=0	**Case 15** Two class n=60, c=0



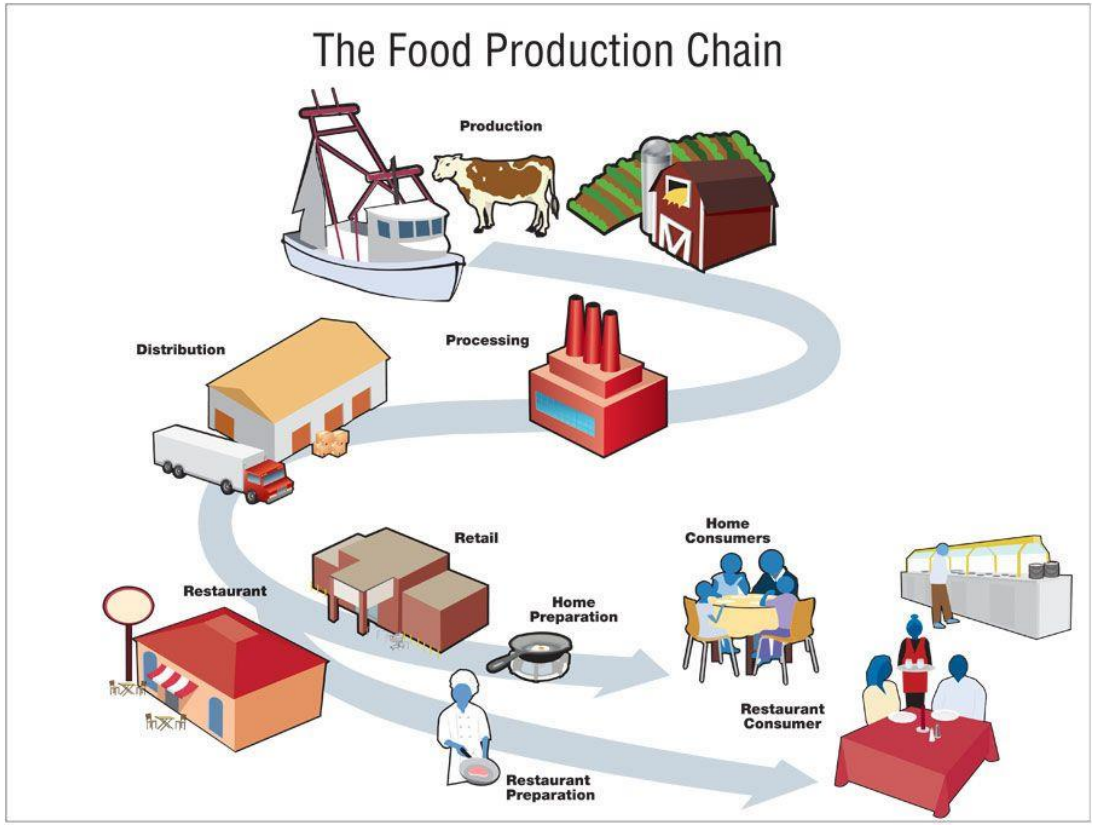
Interrelationship between n and c for microbiological specification stringency

Example of microbiological criteria and sampling plans

Taken from US-Codex Committee on Food Safety

EXAMPLE: MILK POWDER
(point in the food chain where MC is applied)

- Milk powder for import/export inspection by national authorities - verification of lot acceptability
- Milk powder for lot acceptance by food business operators - purchasing from a supplier
- Milk powder at point of manufacture as a verification of process control

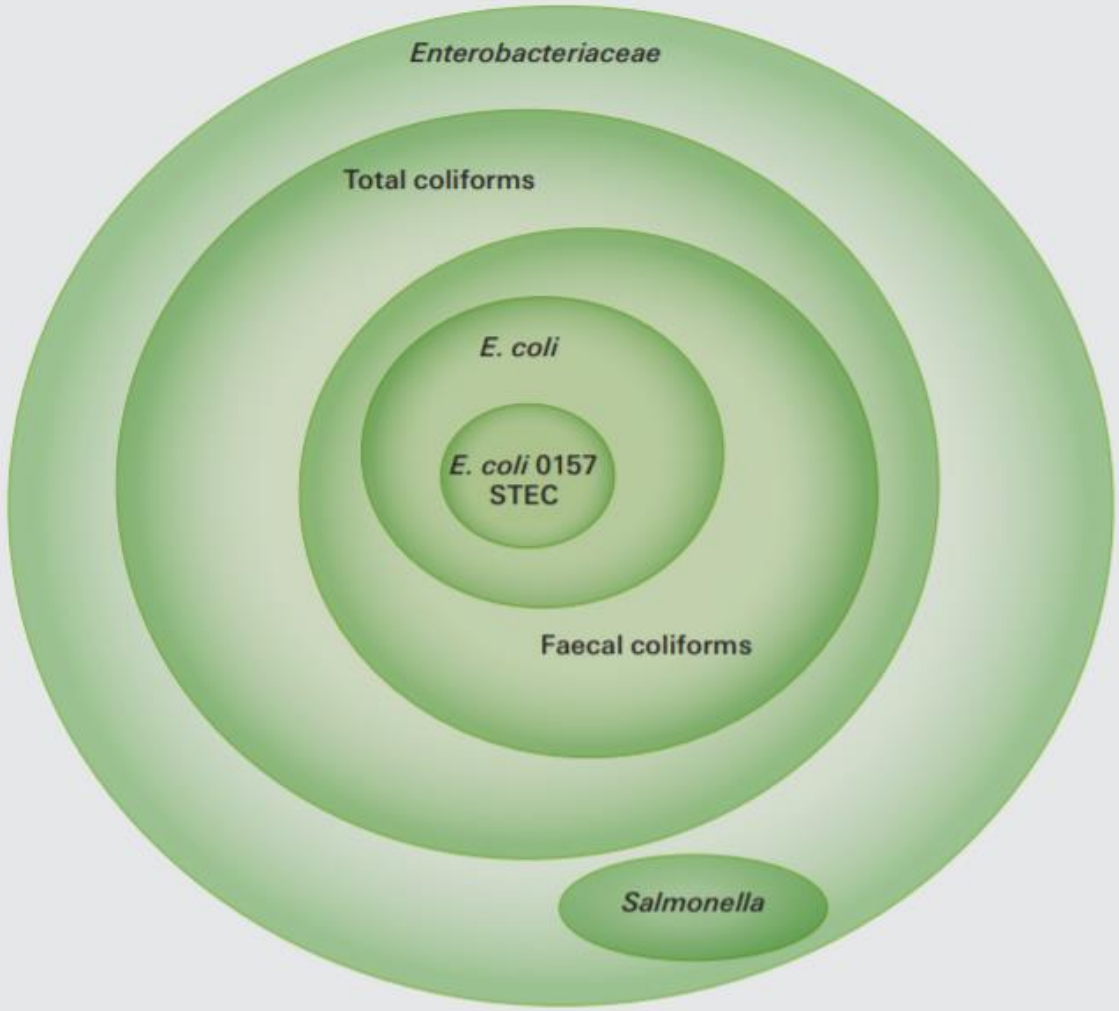


Organism of concern

- Hygiene Criteria:
  - Mesophilic aerobic microorganisms (TPC)
  - Enterobacteriaceae (Coliforms, fecal coliforms)

- Food Safety Criterion
  - *Salmonella* spp.

The relationship between *Salmonella*, pathogenic *E. coli* (*E. coli* O157/STEC) and the commonly used *Enterobacteriaceae* hygiene indicator, and related hygiene indicators



Rationale for the organisms selected (food safety)

- Epidemiological data suggest that the only significant hazard to be controlled during manufacturing of dried products is *Salmonella.*
- *Staphylococcus aureus* or *Bacillus cereus* are only present sporadically at very low levels or occur as a result of major breakdowns of GHP.
- Mesophilic Aerobic Microorganisms - indicators of general contamination, shelf life or spoilage; not usually related to a health hazard
- Enterobacteriaceae – high numbers frequently indicate inadequacy of general hygiene

n: number of sample unit from a lot

c: max acceptable number of sample that exceed microbiological criteria

m: max number of bacteria per gram

M: quantity to prepare acceptable quality vs unacceptable quality (max permitted level)

Sampling Plan

Organism	Analytical unit	n	c	m	M
Mesophilic Aerobic Microorganisms	10 g	5	2	10^4 CFU/g	10^5 CFU/g
Enterobacteria-ceae	10 g	5	2	< 3 MPN/g	9.4 MPN/g
Salmonella spp.	25 g	10	0	Not detected in 25 g	

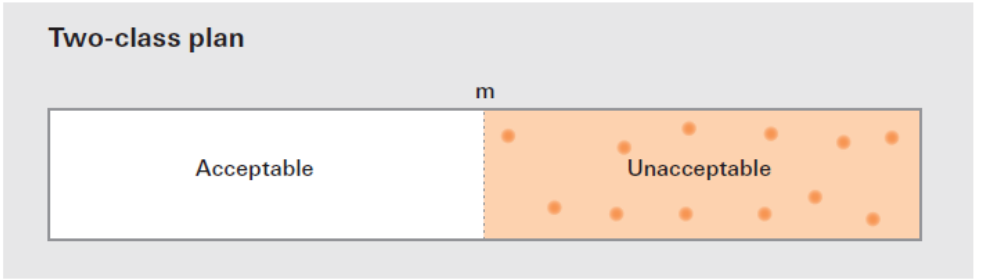
METHODS OF ANALYSIS

Mesophilic aerobic colony count: ISO 4833

Enterobacteriaceae: ISO 21528-1 (MPN technique)

Salmonella spp.: ISO 6785 (Milk and milk products -- Detection of Salmonella spp.) or ISO 6579 (Horizontal method for the detection of Salmonella spp.)

Interpretation of results



The microbiological limit (m) separates the quality of a lot between acceptable and unacceptable in a two-class plan

Salmonella

- Satisfactory if all values observed indicate the absence of the bacterium; the lot is accepted
- Unsatisfactory if the presence of the bacterium is detected in any of the sample units; the lot is rejected

Organism	Analytical unit	n	c	m	M
Mesophilic Aerobic Microorganisms	10 g	5	2	10^4 CFU/g	10^5 CFU/g
Enterobacteria-ceae	10 g	5	2	< 3 MPN/g	9.4 MPN/g
Salmonella spp.	25 g	10	0	Not detected in 25 g	

n: number of sample unit from a lot

c: max acceptable number of sample that exceed microbiological criteria

m: max number of bacteria per gram

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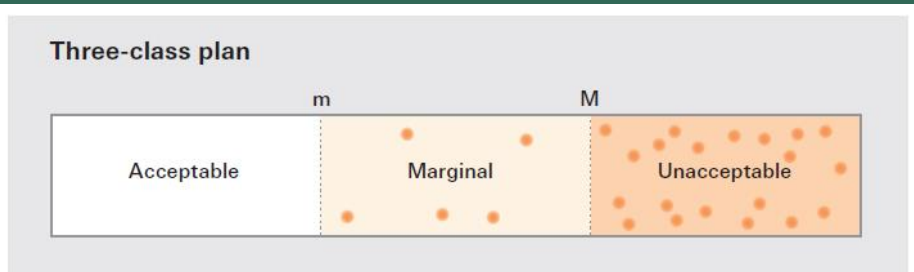
Interpretation of results

Mesophilic aerobic colony count and/or Enterobacteriaceae

- Satisfactory, if all the values observed are ≤ m; the lot is accepted
- Acceptable, if a maximum of c units (2) have values that are between m and M and the rest of the values observed are ≤ m; the lot is accepted
- Unsatisfactory if one or more of the values observed is > M or more than c units (2) are between m and M; the lot is rejected



The microbiological limits (m and M) separate the quality of a lot between acceptable, marginally acceptable and unacceptable in a three-class plan

Organism	Analytical unit	n	c	m	M
Mesophilic Aerobic Microorganisms	10 g	5	2	10^4 CFU/g	10^5 CFU/g
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Salmonella spp.	25 g	10	0	Not detected in 25 g	

n: number of sample unit from a lot

c: max acceptable number of sample that exceed microbiological criteria

m: max number of bacteria per gram

M: quantity to prepare acceptable quality vs unacceptable quality (max permitted level)

Actions in case of non-compliance (Food business operators)

***Salmonella* criterion**	***Salmonella* criterion**
Food business operators purchasing from a supplier 1. Notify supplier 2. Do not use the milk powder 3. If the product has been shipped, recall the product 4. Determine appropriate steps with respect to the supplier	Food business operators manufacturing the milk powder 1. prevent the affected lot from being released for human consumption 2. recall the product if it has been released for human consumption 3. determine and correct the root cause of the failure.

Actions in case of non-compliance (National authorities)

Salmonella criterion

- Prevent the affected lot from being released for human consumption
- Ensure recall of product if it has been released for human consumption
- Reject lot at port of entry.

Actions in case of non-compliance (Food business operators)

Mesophilic aerobic microorganisms and/or Enterobacteriaceae	**Mesophilic aerobic microorganisms and/or Enterobacteriaceae**
Food business operators purchasing from a supplier 1. Notify the supplier 2. Determine appropriate disposition of the non-compliant lot (e.g., refuse lot or accept marginal quality lot, depending on business contractual arrangements)	Food business operators manufacturing the milk powder 1. Check on the efficacy of heat treatment and controls for prevention of recontamination 2. Determine and correct the root cause of the failure and 3. Review and revise monitoring procedures, environmental surveillance and prerequisite programs.

Actions in case of non-compliance (National authorities)

Mesophilic aerobic microorganisms and/or Enterobacteriaceae

1. Reject lot at port of entry (destroy at port of entry or return to country of origin)
2. Notify the manufacturing facility to take corrective actions with respect to hygiene practices and to verify the efficacy of heat treatment and procedures to prevent recontamination
3. Reject lot as part of domestic food control procedures; allow food business operator to destroy or rework

Thank You