



General Principles for Microbiological Sampling and Testing and ICMSF Recommendations for Poultry Products

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Acknowledgments



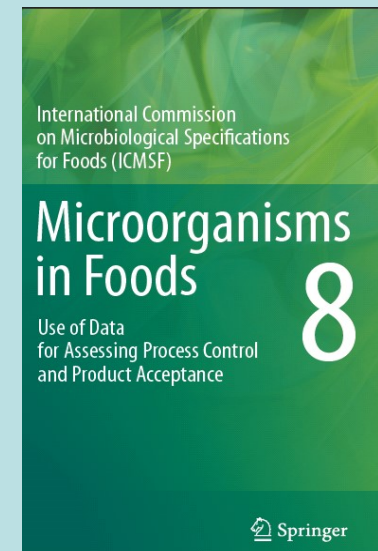
Dr Wayne Anderson, Ireland
Dr Leon Goris, The Netherlands



Presentation Out-line

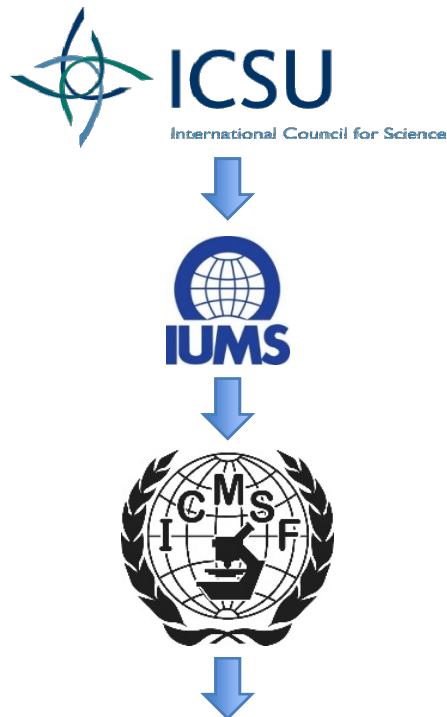


- Introduction-ICMSF
- General Principles of Microbiological sampling and testing
- Examples from poultry chapter
 - Raw poultry meat
 - Dried poultry meat
 - Codex Guidelines/MRAs



ICMSF

International Commission on Microbiological Specifications for Foods



ICMSF meets annually as a working party since 1962



51 meetings in 28 countries



1969





2018



ICMSF's mission

Be a leading source for independent and impartial scientific concepts, that when adopted by governmental agencies and industry, will reduce the incidence of microbiological food-borne illness and food spoilage worldwide and facilitate global trade.



About the ICMSF

- 18 food microbiologists from 14 different countries
- Broad professional backgrounds
- Selected on technical expertise, not as national delegates
- Complemented by an extensive network of other experts engaged as sub-commissions, working groups, consultants, reviewers, collaborators....
- All work is voluntary and without honoraria
- ICMSF recommendations have no official status



Evolution of Food Safety Management



1960s – 1980s
Methods and Testing



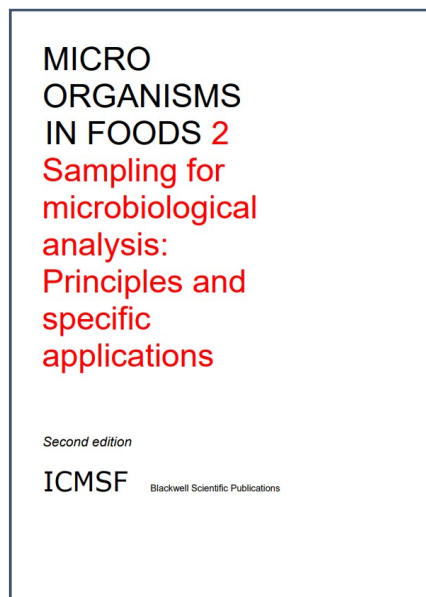
1980s-2000s
Microbial Ecology
Hygiene & HACCP



2000s-2020s
Risk Management



Microbiological Criteria



1st Edition, 1974
2nd Edition, 1986

- Concept first introduced in ICMSF Book 2
- To manage safety and suitability of food in trade
- Concept recommends **15 Cases**
 - Different sampling plan
 - To verify food lot acceptance
- Follows a risk-based approach

Rationale

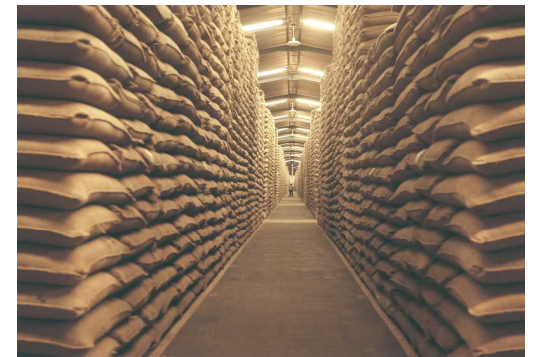
- The **greater the risk**, the **more stringent the management** of the hazard needs to be
- Thus **sampling plans** have been selected with proportionally **higher performance**



Lot acceptance

A food lot represents a unit produced under uniform conditions

- Sampling plans with performance proportional to target microorganism or hazard are used to determine whether a lot of food is acceptable



ICMSF Cases

The 15 cases reflect the relative risk

Hazard:

- *Severity*

- *Level*

- Considering:

- Harmfulness of the microorganism/hazard
- Intended consumer population
- Conditions of food handling and use



ICMSF Categories of Microorganisms/hazards

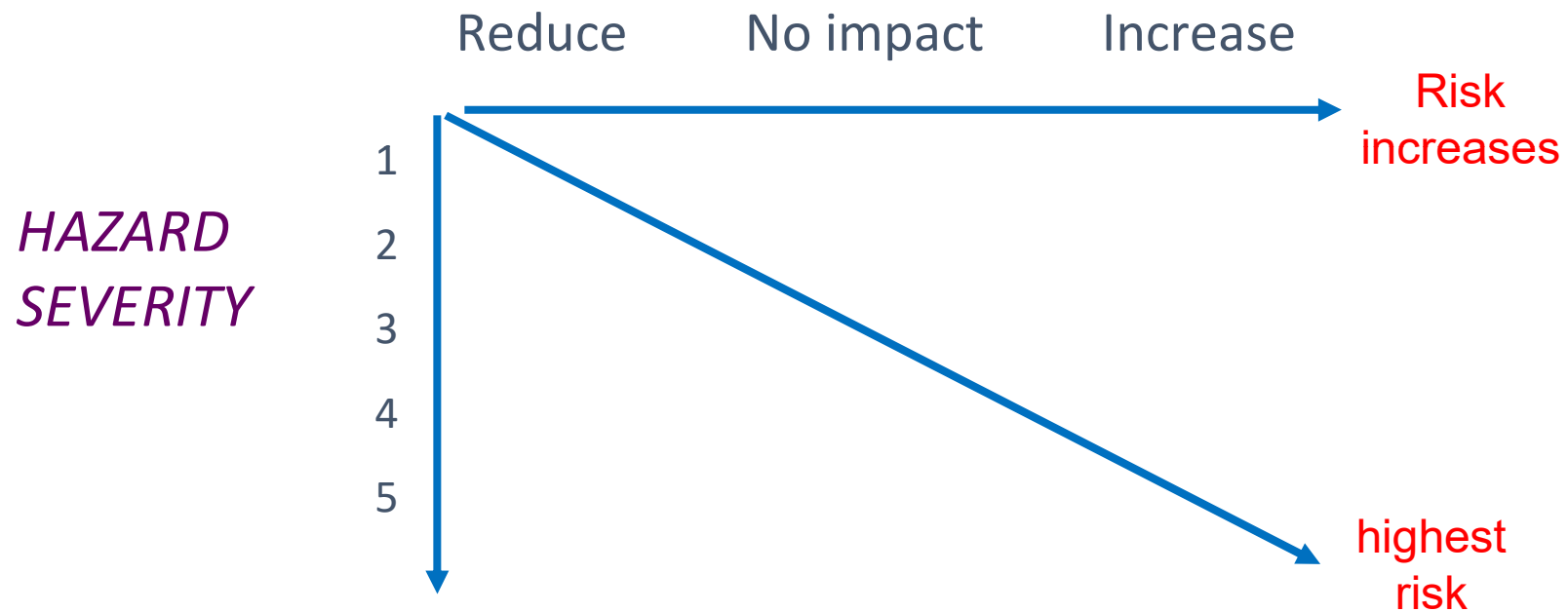
Severity ↓

Utility	Spoilage, reduced shelf life, no health concern	e.g. total counts (TVC, etc.), yeast and mold
Indicator	Measure of GHP	e.g. Coliforms, Enterobacteriaceae
Moderate hazard	Not life threatening, short duration, self limiting, no sequelae	e.g. <i>S. aureus</i> , <i>B. cereus</i> , <i>C. perfringens</i> , Norovirus
Serious hazard	Incapacitating, usually not life threatening	e.g. Salmonellae, <i>Shigella flexneri</i> , <i>Yersinia enterocolitica</i>
Severe hazard	Life threatening, chronic sequelae, or long duration or designed for sensitive sub-population	e.g. <i>E. coli</i> O157:H7, <i>C. botulinum</i> toxin or <i>Cronobacter</i> (infants)



Risk Categorization Matrix

*Impact of food handling and use conditions on
HAZARD LEVEL*



Sampling Plans for Food Lot Acceptance

Microorganism Category	Likely Change in Level in Food Before Consumption		
	<i>Reduce</i>	<i>No Change</i>	<i>Increase</i>
1	Case 1	Case 2	Case 3
2	Case 4	Case 5	Case 6
3	Case 7	Case 8	Case 9
4	Case 10	Case 11	Case 12
5	Case 13	Case 14	Case 15



Sampling Plans for Lot acceptance

Likely Change Before Consumption

Category	Reduce	No Change	Increase	
Utility	Case 1 n=5, c=3	Case 2 n=5, c=2	Case 3 n=5, c=1	} Class 3 sampling plans
Indicator	Case 4 n=5, c=3	Case 5 n=5, c=2	Case 6 n=5, c=1	
Moderate	Case 7 n=5, c=2	Case 8 n=5, c=1	Case 9 n=10, c=1	
Serious	Case 10 n=5, c=0	Case 11 n=10, c=0	Case 12 n=20, c=0	} Class 2 sampling plans
Severe	Case 13 n=15, c=0	Case 14 n=30, c=0	Case 15 n=60, c=0	



Relative performance of ICMSF cases*

	Conditions under which food is expected to be handled and consumed after sampling in the usual course of events		
	Reduce risk	No change in risk	May increase risk
Utility	Case 1 3-class: n = 5, c = 3, m = 1000/g, M= 10000/g <u>Mean conc.: 5105/g</u>	Case 2 3-class: n = 5, c = 2, m = 1000/g, M= 10000/g <u>Mean conc.: 3282/g</u>	Case 3 3-class: n = 5, c = 1, m = 1000/g, M= 10000/g <u>Mean conc.: 1829/g</u>
Indicator	Case 4 3-class: n = 5, c = 3, m = 100/g, M= 1000/g <u>Mean conc.: 511/g</u>	Case 5 3-class: n = 5, c = 2, m = 100/g, M= 10000/g <u>Mean conc.: 328/g</u>	Case 6 3-class: n = 5, c = 1, m = 100/g, M= 10000/g <u>Mean conc.: 183/g</u>
Moderate hazard	Case 7 3-class: n = 5, c = 2, m = 10/g, M= 100/g <u>Mean conc.: 33/g</u>	Case 8 3-class: n = 5, c = 1, m = 10/g, M= 100/g <u>Mean conc.: 18/g</u>	Case 9 3-class: n = 10, c = 1, m = 10/g, M= 100/g <u>Mean conc.: 6/g</u>
Serious hazard	Case 10 2-class: n = 5, c = 0, m = 0/25g <u>Mean conc.: 1/55g</u>	Case 11 2-class: n = 10, c = 1, m = 0/25g <u>Mean conc.: 1/178g</u>	Case 12 2-class: n = 20, c = 1, m = 0/25g <u>Mean conc.: 1/495g</u>
Severe hazard	Case 10 2-class: n = 15, c = 0, m = 0/25g <u>Mean conc.: 1/328g</u>	Case 11 2-class: n = 30, c = 1, m = 0/25g <u>Mean conc.: 1/854g</u>	Case 12 2-class: n = 60, c = 1, m = 0/25g <u>Mean conc.: 1/2034g</u>



* illustrated in terms of the mean concentration that will be rejected with at least 95% probability, assuming hypothetical criteria and a standard deviation of 0.8. Calculations were performed with ICMSF Microbiological Sampling plan tool Version 2.08 (www.icmsf.org).

Example: Cooked poultry Product-End Product Testing to verify lot acceptance (1)

Product	Microorganism	Analytical method	Sampling plan & limits/g				
			Case	n	c	m	M
Cooked poultry	<i>Staph aureus</i>	ISO 6888-1	8	5	1	10^2	10^3

Meaning:

Case 8: Moderate hazard, level No change

$n = 5$: take five samples and enumerate the *staph aureus* concentration

$m = 10^2$: start of the marginally acceptable concentration (100 cfu/g)

$M = 10^3$: maximum marginally acceptable concentration (1000 cfu/g)

$c = 1$: maximum three samples can be between 100 and 1000 cgu/g
no samples are allowed to be over 1000 cfu/g



Example: Cooked Product-End Product Testing to verify lot acceptance (2)

Product	Microorganism	Analytical method	Sampling plan & limits/25g				
			Case	n	c	m	M
Cooked poultry	<i>Salmonella</i>	ISO 6579	11	10	0	0	

Meaning:

Case 11: Severe pathogen, level may stay unchanged

$n = 10$: take thirty samples of 25g

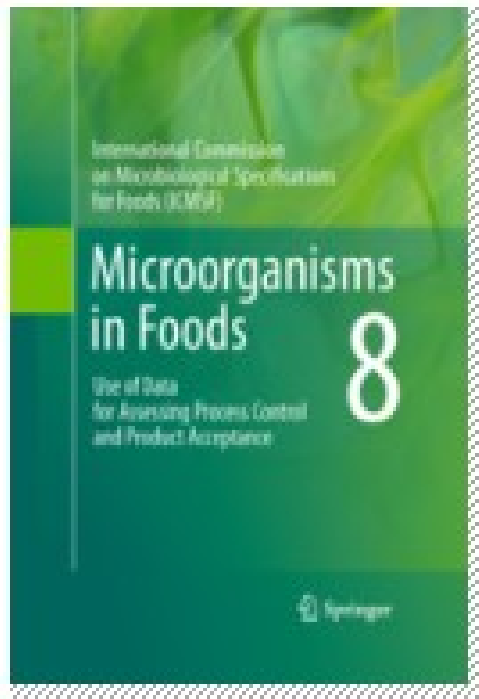
m = *Salmonella* should not be detected in 25g

$c = 0$: none of the 30 samples can be positive for O157:H7





ICMSF Advice / Recommendations




Microorganisms in Foods 8

Use of Data for Assessing Process Control and Product Acceptance
International Commission on Microbiological Specifications for
Foods (ICMSF)

2011, 2011, XX, 400 p. 12 illus.

Available Formats:

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<https://www.springer.com/gp/book/9781441993731>

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8: Meat Products

9: Poultry Products

10: Fish and Seafood Products

11: Feeds and Pet Food

12: Vegetables and Vegetable Products

13: Fruits and Fruit products

14: Spice, Dry Soups and Asian Flavourings

15: Cereals and Cereal Products

16: Nuts, Oilseeds, Dried Legumes and Coffee

17: Cocoa, Chocolate and Confectionery

18: Oil- and Fat-Based Foods

19: Sugar, Syrups and Honey

20: Non-alcoholic Beverages

21: Water

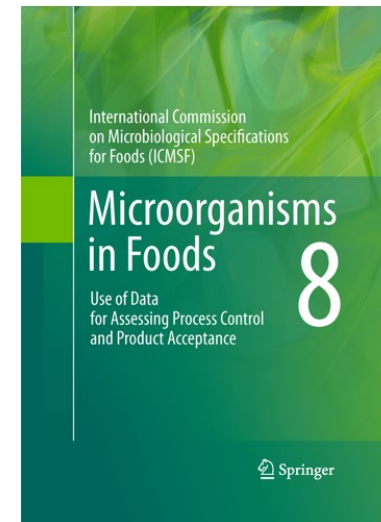
22: Eggs and Egg Products

23: Milk and Dairy Products

24: Shelf-Stable Heat Treated Foods

25: Dry Foods for Infants and Young Children

26: Combination Foods



Part II

Application of Principles to Product Categories

Chapter 9: Poultry Products pg 95-106





Chapter 9: Poultry Products



Contents

- Raw poultry products
- Cooked poultry products
- Fully retorted shelf-stable poultry products
- Dried Poultry Products



Category: Sub-headings



- Significant organisms
- Relative importance and usefulness of data on Microbiological testing
 - Critical ingredients
 - In-process
 - Processing environment
 - Shelf life
 - End product



Poultry Products



Primary production

- Small farms, Extensive production
 - Control programmes on farm difficult
- Large farms, intensive indoor production
 - Control programmes for *Salmonella* and *Campylobacter* can be realised





Raw Poultry Products



Examples

- Whole Chicken
- Chicken portions/pieces

Main Pathogens

- Salmonella
- Campylobacter





Raw Poultry Product Testing



Relative importance		Useful testing
Critical ingredients	Low	Time and temperature should be controlled for raw poultry ingredients. Routine testing of non-meat ingredients, if any, is not recommended
In-process	Medium	Test whole carcass rinse or tissue samples (e.g. neck flap) to establish a baseline at various stages of processing and to evaluate where changes in the microbial populations occur during processing. Typical levels for psychrotrophs, <i>E. coli</i> and <i>Salmonella</i> depend on sampling site , sampling method and processing conditions within each factory.
Processing environment	Medium	Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting. Typical levels encountered <500 cfu/cm ² but may vary by surface type.
Shelf life	Low	Routine shelf life testing of refrigerated product is not recommended . Testing of frozen product is also not recommended . <i>Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are installed.</i>



Raw Poultry Testing.....contd.



Relative importance

Useful testing

End product

Medium

Test for indicator organisms for on-going process control and trend analysis of fresh pack product using internally developed guidelines. Typical levels encountered:

- Aerobic colony count $<10^5$ CFU/g
- *E.coli* $<10^2$ CFU/g

Not usually applicable to retail product or product in the distribution chain.

Routine lot acceptance sampling is not recommended for salmonellae or *Campylobacter* on raw poultry. Outbreak investigations or new supplier certification may benefit from determining the prevalence of salmonellae or *Campylobacter* in some situations

- In countries or regions that have established performance objectives, the required sampling plan and tests should be applied



Cooked Poultry Products



- **Perishable**

- Stored frozen or chilled

- **Examples**

- Cooked sliced chicken and turkey
- Cooked chicken tikka pieces
- Cooked portions of chicken, turkey or duck

- **Main Pathogens**

- Salmonella
- Campylobacter
- *Listeria monocytogenes*
- *Clostridium perfringens*
- *Staphylococcus aureus*





Cooked Poultry Product Testing



Relative importance		Useful testing
Critical ingredients	Low	These products do not contain non-poultry ingredients of significance for microbiological safety or quality
In-process	High	Monitoring the cooking parameters is essential
	Medium	For product that supports <i>L.monocytogenes</i> growth , post -cook samples can assess control of <i>Listeria spp.</i> <i>Listeria spp.</i> should be absent
Processing environment	High	For products that support the growth of <i>L.monocytogenes</i> : During production sample product contact surfaces before packaging after cooking (where product exposed). Sponge or swab samples from floors and drains and other non-product contact surfaces for an early indication of the level of control. <i>Listeria spp.</i> should be absent
	Medium	Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfection procedures. Typical levels encountered <500 cfu/cm ² but may vary by surface type
Shelf life	Medium	Shelf life testing may be useful for chill products with extended shelflife but not frozen product



Cooked Poultry Product Testing.....contd.



Relative

importance

Useful testing

End
produ
ct

Medium

Test for indicators of ongoing process control and trend analysis. Typical levels $ACC 10^4$ cfu/cm²; *E. coli* absent

Sampling plan & limits/g

Product	Microorganism	Analytical method	Cas e	n	c	m	M
Cooked poultry (possible temperature abuse)	<i>S. aureus</i>	ISO 6888-1	8	5	1	10 ²	10 ³
Cooked poultry: no growth	<i>L. monocytogenes</i>	ISO 11290-2	N/A	5	0	10 ²	-
Cooked uncured poultry	<i>C. perfringens</i>	ISO 7937	8	5	1	10 ²	10 ³



Cooked Poultry Product Testing.....contd.

Relative importance Useful testing (continued)

End
product

Product	Microorganism	Analytical method	Sampling plan & limits/25g				
			Cas e	n	c	m	M
Cooked poultry	<i>Salmonella</i>	ISO 6579	11	10	0	0	-
Cooked poultry: supports growth	<i>L. Monocytogenes</i>	ISO 11290-1	N/A	5	0	0	-



Plan Performance Examples: cooked meat

- Geometric mean concentration (per g or ml) at 95% probability of rejection (**table A3 pg 362**)
 - PLAN: *L.monocytogenes* **n=5 c=0 m=0 (25g)**
 - » S.d.(0.25) = 1 cell in 44g; S.d. (0.5) = 1 cell in 49g;
 - » S.d.(0.8) = 1 cell in 55g; S.d. (1.2) = 1 cell in 62g



Dried Poultry Products

- Examples
 - Dried cooked chicken
 - Bouillon powder/paste
 - Dried salted chicken strips
- Main Pathogens
 - *Salmonella*





Dried Poultry Product Testing - I



Relative importance		Useful testing
Critical ingredients	Low	These products do not contain non-meat ingredients of significance for microbiological safety or quality
In-process	High	Monitoring the cooking and formulation parameters like pH and preservatives. The manufacturing process should be validated for control of salmonellae that are present in poultry meat
	Low	Routine microbiological testing of in-process samples is not recommended
Processing environment	Medium	Sample equipment surfaces before startup to verify cleaning and disinfection . E.g. ACC typical levels encountered <500 cfu/cm² but may vary by surface type.
Shelf life	Low	These products are inherently shelf-stable when properly dried and protected from high humidity. The higher the a_w of snack products may require verification of stability e.g. mould



Dried Poultry Product Testingcontd

Relative importance

End product

Medium

Useful testing

Routine sampling is not necessary. If application of **GHP and HACCP is in question**, sampling for Salmonellae may be considered.

Product	Microorganism	Analytical method	Sampling plan & limits/25 g				
			Case	n	c	m	M
Dried Poultry	<i>Salmonella</i>	ISO 6579	11	10	0	0	-



Plan Performance : Dried Poultry Products

- Geometric mean concentration (cfu per g) at 95% probability of rejection (**Table A3, pg 362**)
 - PLAN: *Salmonella* **$n=10$ $c=0$ $m=0$ (25g)**
 - » S.d.(0.25) = 1 cell in 93g; S.d. (0.5) = 1 cell in 120g;
 - » S.d.(0.8) = 1 cell in 180g; S.d. (1.2) = 1 cell in 310g



Global authority for international guidelines, standards,
and recommendations on food safety

<http://www.fao.org/fao-who-codexalimentarius/en/>



Codex

CAC/RCP 58-2005

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CODE OF HYGIENIC PRACTICE FOR MEAT¹

CAC/RCP 58-2005

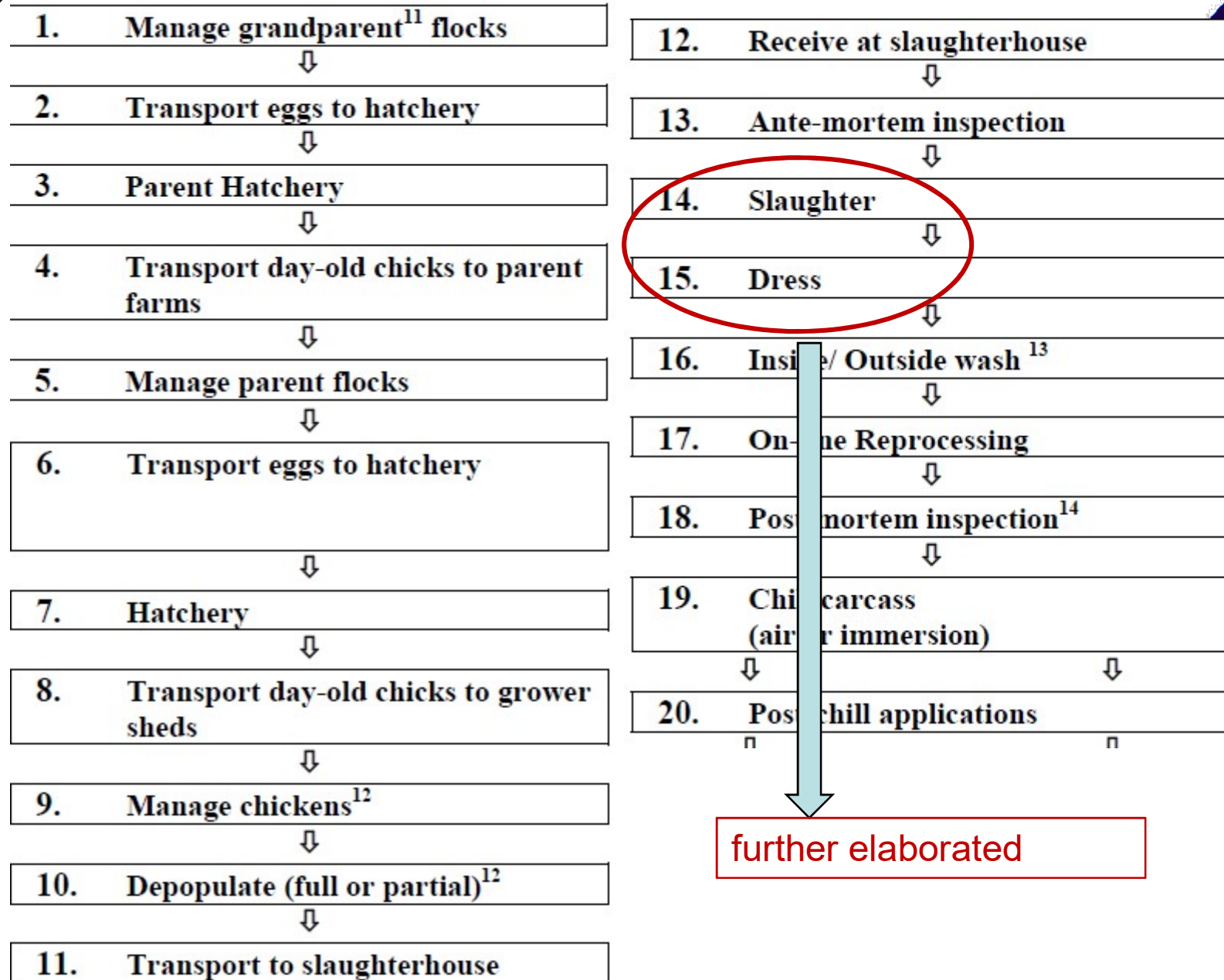
1. INTRODUCTION	3
2. SCOPE AND USE OF THIS CODE	3
3. DEFINITIONS	4
4. GENERAL PRINCIPLES OF MEAT HYGIENE	8
5. PROGRAM PROVISIONS	0

GUIDELINES FOR THE CONTROL OF *CAMPYLOBACTER* AND *SALMONELLA* IN CHICKEN MEAT

CAC/GL 78-2011



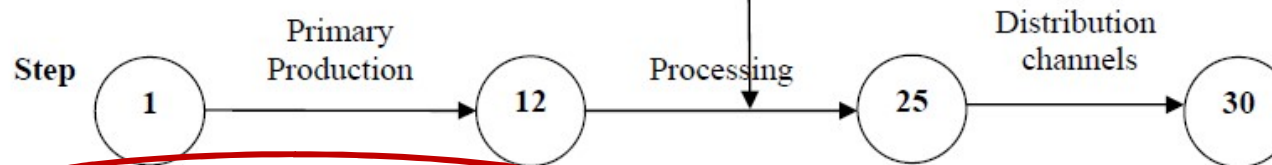
Primary production to Consumption





9.5

Step 16: Inside/outside wash



9.5.1 GHP-based control measures

66. The inside and outside of all carcasses should be thoroughly washed, using pressure sufficient to remove visible contamination. Appropriate equipment should be used to ensure direct water contact with the carcass. The removal of contaminants may be aided by the use of brushing apparatus installed in line with the inside/outside wash.

9.5.2 Hazard-based control measures

For *Campylobacter*

67. Carcass washing systems with 1-3 washers using water with 25-35ppm total chlorine have been shown to reduce levels of *Campylobacter* by about 0.5 log₁₀ CFU/ml of whole carcass rinse sample. Post-wash sprays using Acidified Sodium Chlorite (ASC) or TSP may further reduce *Campylobacter* levels by an average of 1.3 log₁₀ CFU/ml or 1.0 log₁₀ CFU/ml of whole carcass rinse sample respectively.

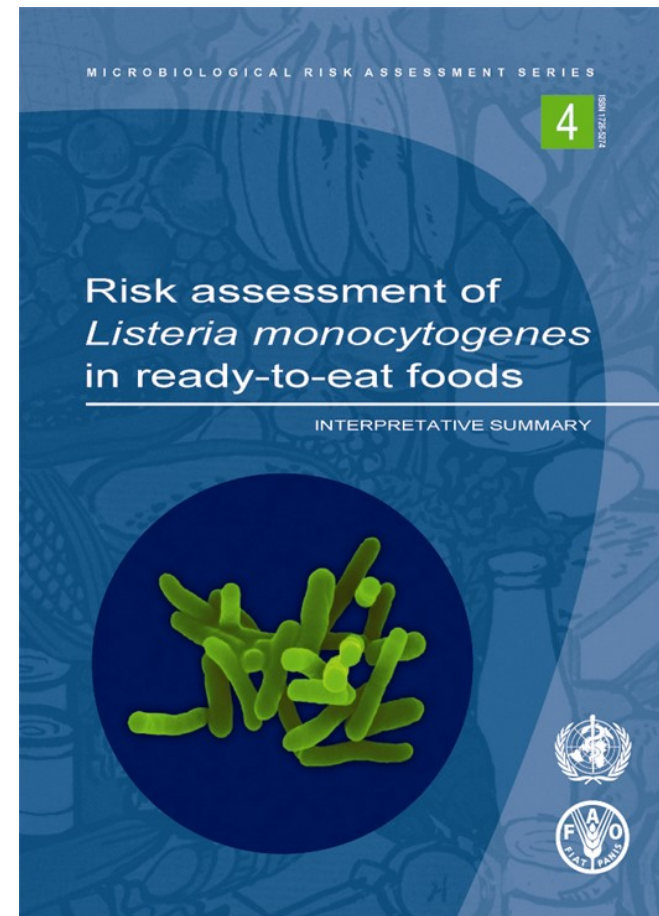
For *Salmonella*

68. Inside/outside washing using a spray application of 20-50 ppm chlorinated water has been shown to reduce the prevalence of *Salmonella*-positive broiler carcasses from 25% to 20%. A second inside/outside washing following upon the first resulted in a reduction of *Salmonella*-positive broiler carcasses from 16%

- GHP based-controls
- Hazard-based controls

Codex guidance for control of *Listeria monocytogenes* in RTE food based on MRA

- **Some insights from MRA study:**
 - Vast majority of listeriosis cases results from ingestion of very high numbers of pathogen
 - Consumption of low numbers of pathogen has a very low probability of causing illness
 - Level of pathogen that is tolerable at the point of consumption is in the order of 100 CFU/g for generally healthy consumers
 - **RTE food products differ in relative presence of the pathogen and their ability to support growth of the pathogen**



Control of *L. monocytogenes* in RTE food Codex guidelines (CAC/GL 61 – 2007)

- Annex II: Microbiological Criteria

foods in which **growth** of *L. monocytogenes* **will not occur**,
i.e. foods that do not support pathogen growth


Foods in which **growth** of *L. monocytogenes* **can occur**,
i.e. foods that do support pathogen growth



A. Foods not supporting growth of *L. monocytogenes*

Rationale:

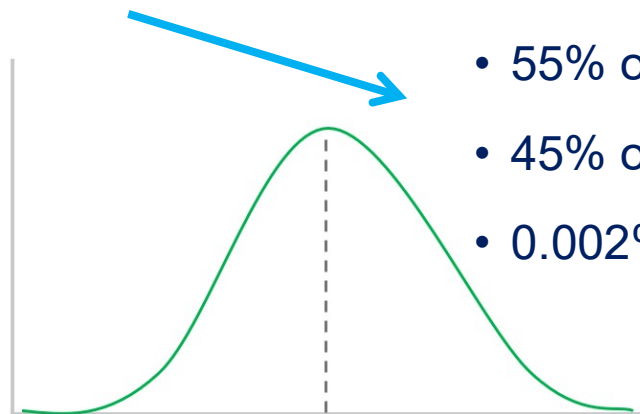
- There is a (low) level of *Lm* that can be considered as “tolerable” for generally healthy consumers.
- Levels of *Lm* very rarely over 1000 CFU/g.
- Unsafe levels for generally healthy consumers occur very, very infrequently



n	c	m	Class Plan
5 ^a	0	100 cfu/g ^b	2 ^c

Micro Criterion performance:

- 55% of samples **below** 100 cfu/g
- 45% of samples **above** 100 cfu/g.
- 0.002% could be **above** 1000 cfu/g.



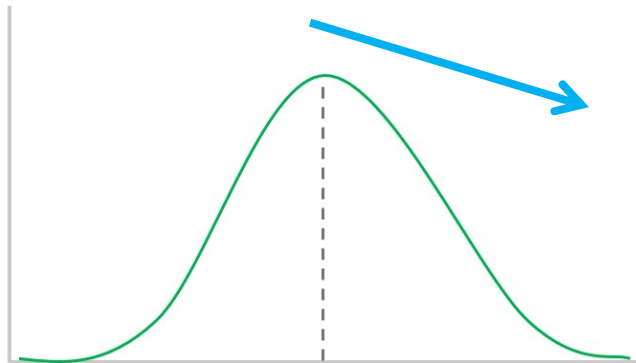
B. Foods supporting growth of *L. monocytogenes*

Rationale:

- Per default, growth is not controlled to any “safe level”
- A large safety margin is needed from levels that are considered unsafe for generally healthy consumers



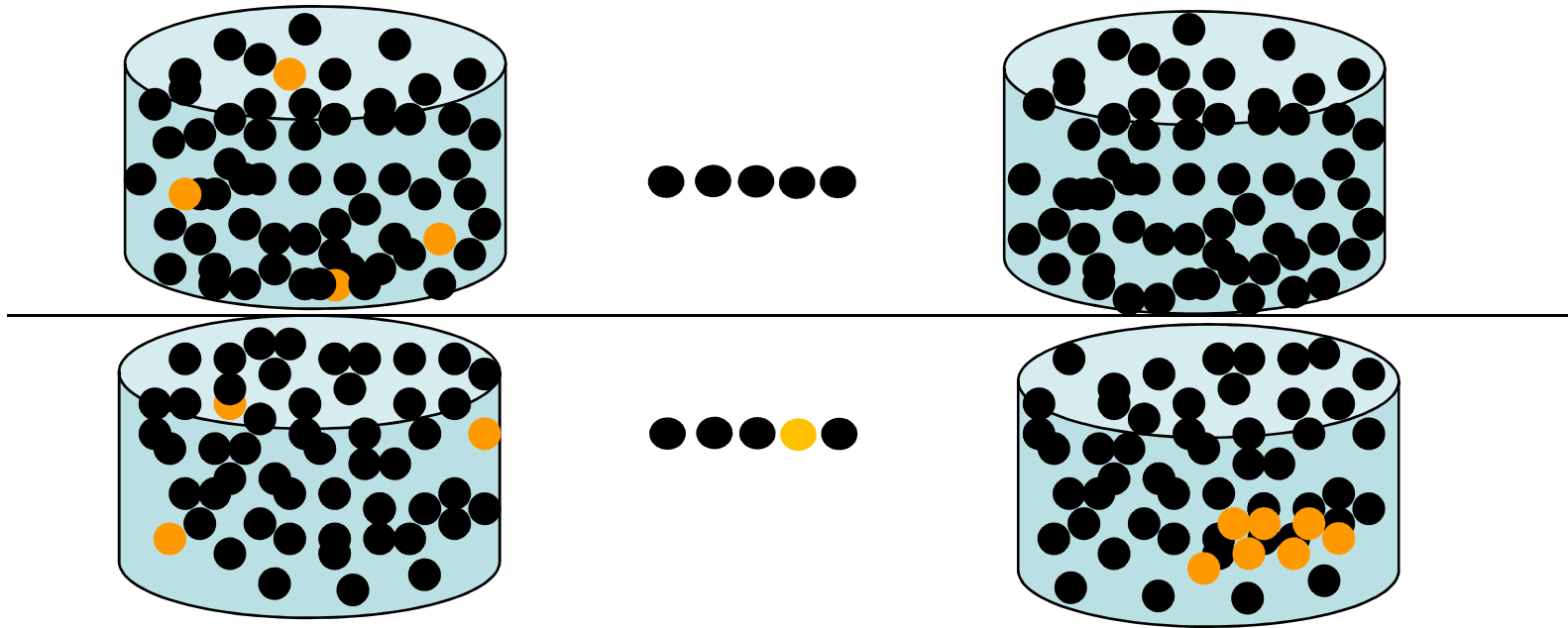
Microorganism	n	c	m	Class Plan
<i>Listeria monocytogenes</i>	5 ^a	0	Absence in 25 g (< 0.04 cfu/g) ^b	2 ^c



Micro Criterion performance:

- 55 % of samples **negative**
- 45 % of sample **positive**.
- 0.5 % could be **above** 0.1 cfu/g.

Limited use of testing for food safety

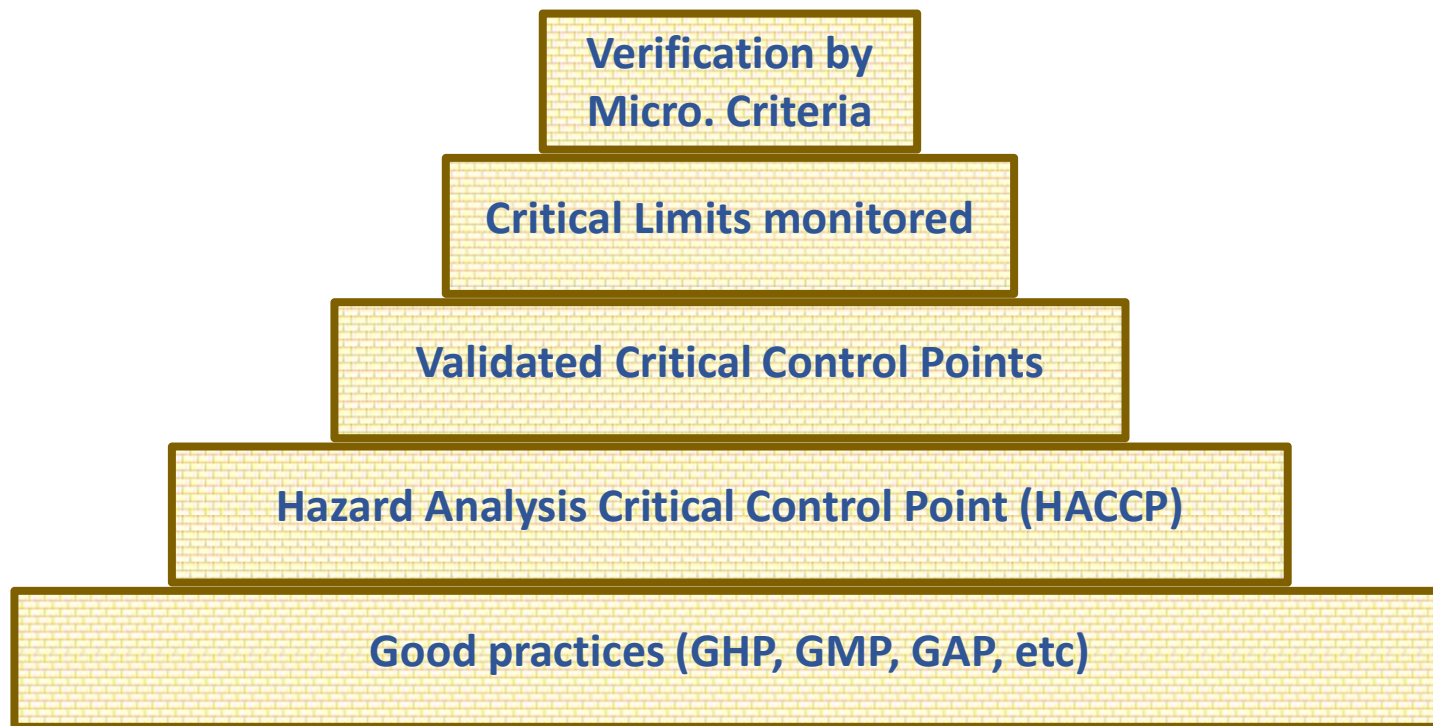


End product testing a lottery ?

Positives mean something, negatives are no guarantee



Evolution: building a strong Food Safety Assurance System for food business operations*



Design a solid food safety system
Use testing to verify that food safety system works properly



Conclusions

- Microbiological testing is an **integral part of food safety** programs, but must be used **appropriately & pragmatically**
- No food safety program can rely solely on microbiological testing
- ICMSF recommendations should **be used as a guideline**
- Legislative microbiological standards in national and international trade need to be applied and complied with
- The **ICMSF sampling plan performance calculator** can be used to **evaluate alternative plans**. (<http://www.icmsf.org/wp-content/uploads/2018/01/Standard-Program.xlsm>)

