

# Microbiology sampling plans



# Microbiological sampling plans

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- ❑ Define the probability of detecting microorganisms in a lot
- ❑ None can ensure the absence of a particular microorganism
- ❑ Should be economically feasible

▶ Sampling for microbiological analysis

▶ sampling plan → quality of batch → release safe food

# Types of microbiological sampling plans

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## ❑ Variables plans

- Non-grouped quantitative analytical results
- Require distributional assumptions to be made (preliminary knowledge about the product)

## ❑ Attributes plans (two- and three-class 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)
- No preliminary idea about MO distribution

**Sampling plans contain the components of microbiological criteria.**

# Choice of a microbiological sampling plan

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Choice depends on

- ❑ The relative degree of risk for the quality of food or the health of the consumer on the basis of the information regarding the product microorganisms
- ❑ The expected number of destroyed, survivors or multiplied organisms during the technological operations to manufacture a food product

# The two-class plan

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- ❑ Samples into one of two classes: **accept** or **reject**
- ❑ Based on presence or absence of an organism or the numbers present
- ❑ Sampling plan is defined by:
  - **n** – the number of sample units to be tested (chosen independently and randomly from the lot)
  - **m** – the count above which the sample is considered defective; if acceptance or rejection is based on presence or absence, then  $m=0$
  - **c** - the maximum number of allowable positives or samples with counts above **m**



# The two-class plan

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**Example:** Criteria for cooked frozen crab meat:

□ *Staphylococcus aureus*       $n=5$        $c=0$        $m = 10^3/g$

If all 5 samples contain  $10^3$  cfu/g or less, the batch is accepted.

If **1** sample has more than  $10^3$  cfu/g, the batch is rejected.

□ *Salmonella spp.*       $n=10$        $c=0$        $m=0$

10 samples of 25 g are tested. If **any** of the samples is positive, the batch is rejected.

# The two-class plan

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Generally used for **pathogens** – three categories:

- ❑ Pathogens presenting **a severe hazard**
  - highly potent toxins with high mortality (*Cl. botulinum*) or
  - organisms causing infection with high mortality, low infective dose and which may spread (*Salmonella typhi*)
  
- ❑ Pathogens presenting **a moderate hazard**
  - with potentially extensive spread (*Salmonella*) (m=0)
  - with limited spread (not necessarily a hazards)
  
- ❑ Two-class plans may be used for indicators as well (*E. coli* as indicator for water supplies or shellfish)

# The three-class plan

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□ Product divided into **three classes** based on the numbers of organisms present

- **0 to m**

- **m to M**

- **above M**

**M** – the count above which the lot is unacceptable

**m** – the count which separates good quality from marginally acceptable quality

**c** – the number of samples with acceptable counts



# The three-class plan

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**Example:** Aerobic plate count for pre-cooked breaded shrimp

$n=5$

$c=2$

$m = 5 \times 10^5/\text{g}$

$M = 10^7/\text{g}$

Sample	Count, cfu/g	Conclusion
1	$6.3 \times 10^6$	Two samples fall between m and M. All other samples are below m. As $c=2$ , the batch is accepted.
2	$4.8 \times 10^5$	
3	$2.1 \times 10^5$	
4	$5.9 \times 10^5$	
5	$3.6 \times 10^5$	

# The three-class plan

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Generally used for

- ❑ Organisms associated with general contamination and spoilage – no hazard to consumer (aerobic plate counts)
- ❑ Indicators – no direct hazard to consumer, but significant numbers may indicate general process hygiene, post-process re-contamination or temperature abuse (Enterobacteriaceae – problems with general hygiene, *E. coli* – faecal contamination)
- ❑ Pathogens representing a moderate hazard with limited or no spread (*Staphylococcus aureus*)

# The three-class plan

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- ❑ **m** – defined by the level of contamination that can be attained by good manufacturing practice
- ❑ **M** – numbers giving rise to detectable spoilage, unacceptably short storage life or a potential hazard

## Aerobic count in pre-cooked breaded shrimps

**n=3**

**c=2**

**m=5x10<sup>5</sup>**

**M=10<sup>7</sup>**

Batch	Sample	Count cfu/g	Conclusion
1	1	4.0 x 10 <sup>4</sup>	MO count in all samples is below m. The batch is <u>accepted</u> .
	2	3.2 x 10 <sup>5</sup>	
	3	4.2 x 10 <sup>5</sup>	
2	1	6.3 x 10 <sup>6</sup>	MO count in two samples is between m и M. In 1 sample it is below m. Because c=2, the batch is <u>accepted</u> .
	2	2.1 x 10 <sup>5</sup>	
	3	5.9 x 10 <sup>5</sup>	
3	1	3.2 x 10 <sup>5</sup>	MO count in 1 sample is above M. The batch is <u>rejected</u> .
	2	7.8 x 10 <sup>7</sup>	
	3	4.8 x 10 <sup>5</sup>	

# Plan stringency

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Decisions should take into account

- ❑ Potential temperature abuse during storage, distribution and retailing
- ❑ Possible abuse by the consumer
- ❑ The target consumer
- ❑ Whether consumer will normally cook the food, type of cooking
- ❑ How much food is expected to be consumed





# Choice of a sampling plan

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- ❑ Purpose of control - acceptance or rejection, quality assessment, determination of uniformity
- ❑ Nature of the lot - size, dividing into sub-lots
- ❑ Nature of test material - uniformity, size of units, price
- ❑ Nature of analytical procedures - destructive or non-destructive, duration and price of analysis

# Sample analysis

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- ❑ Collection of samples
- ❑ Transportation
- ❑ Sample preparation
- ❑ Analysis



# Sample analysis

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- ❑ Liquid samples



- ❑ Samples from solid products

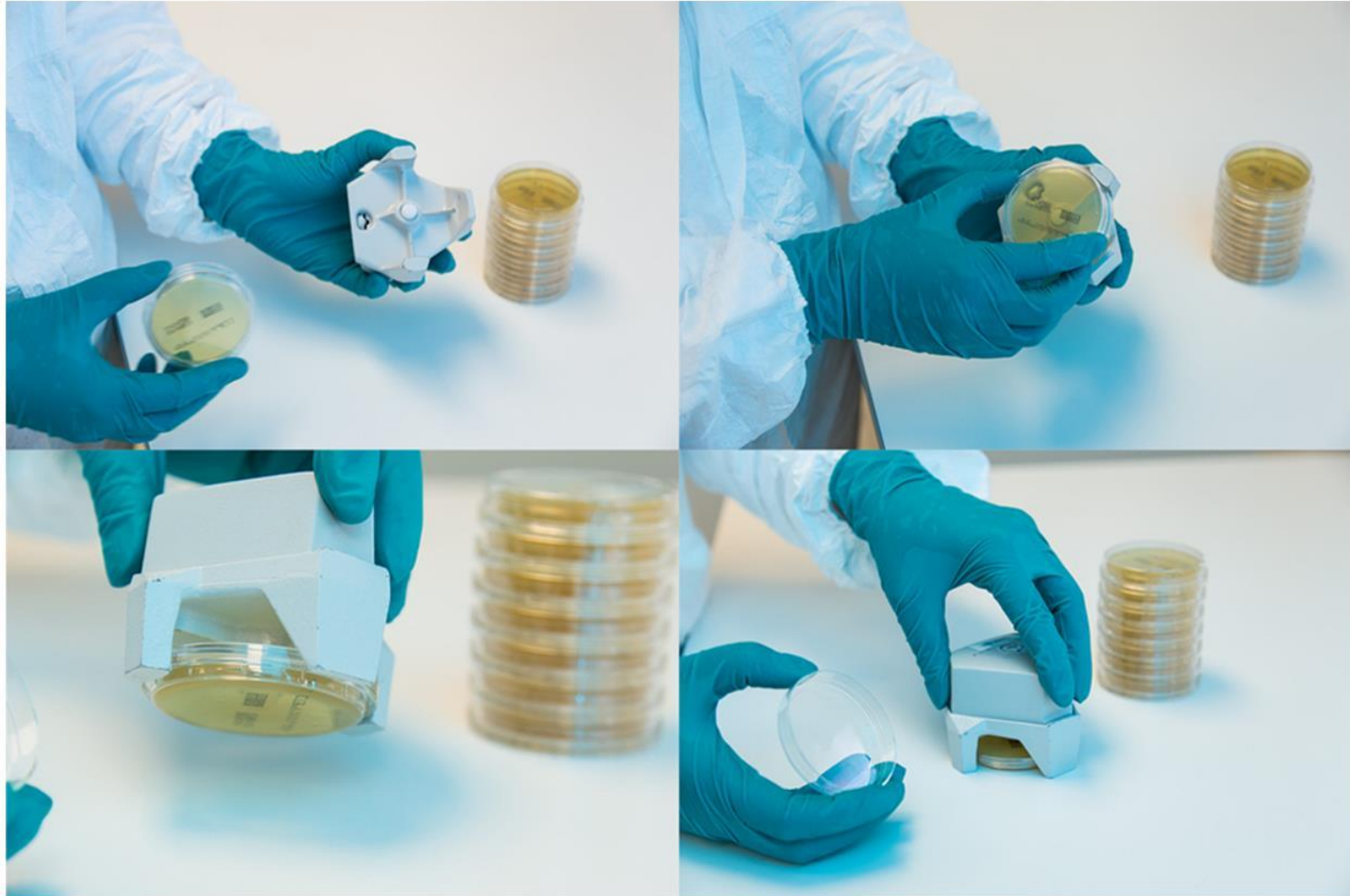


- ❑ Samples from surfaces



# Sample analysis

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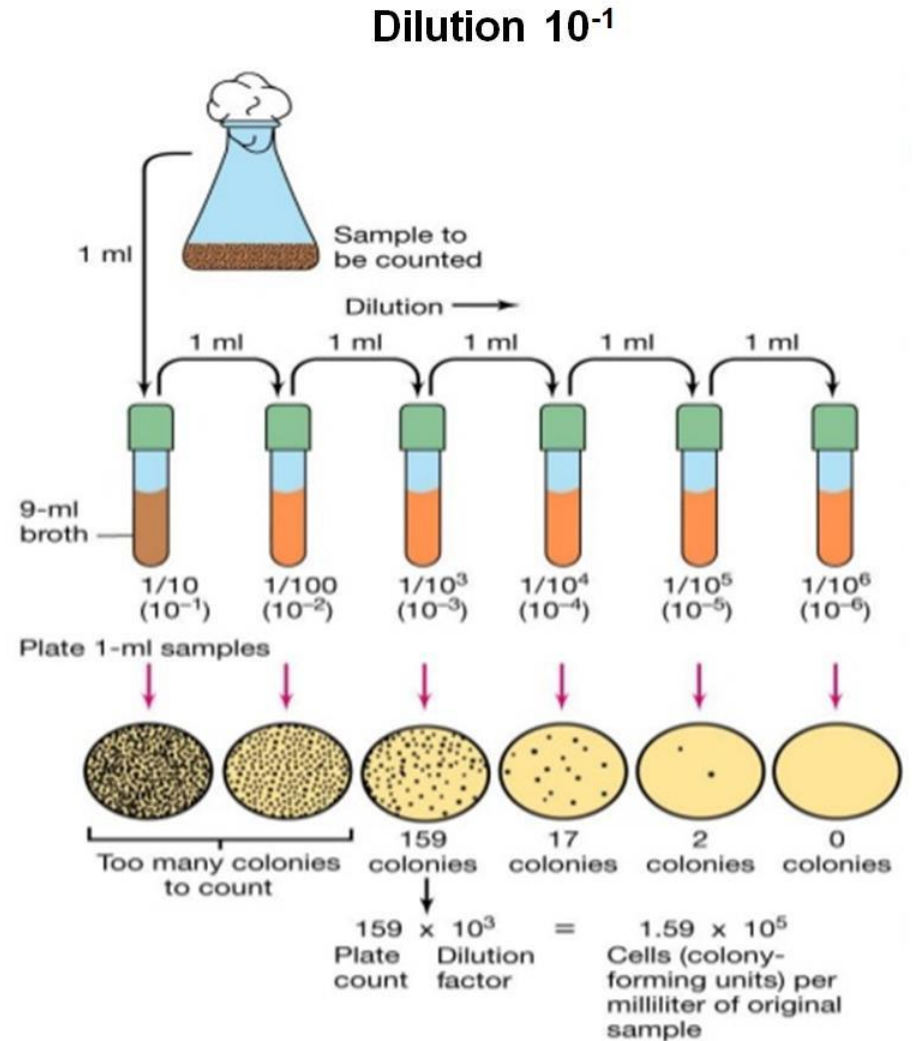
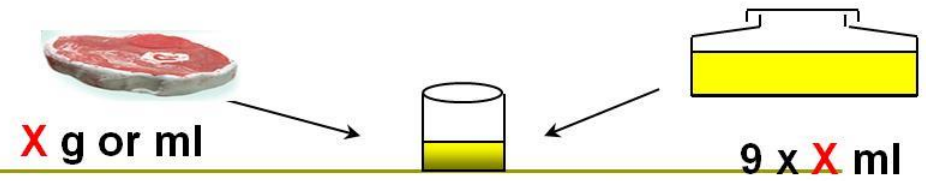
# Sample analysis

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- ❑ Liquid samples – possible dilution, filtration
- ❑ Powder samples - dilution
- ❑ Samples from solid products – homogenization (blender, Stomacher)
- ❑ Choice of dilution solution
  - 0.1% peptone water (pH=6.8-7.0), phosphate buffer or Ringer solution; combination of 0.1% peptone and 0.75% NaCl (ISO 6887:1983)
  - solutions for osmophilic and halophilic microorganisms



# Sample analysis



# Sample analysis

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- ❑ **ISO 7218**: Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations
- ❑ **ISO 6887-1/2/3/4/5/6**: Microbiology of food and animal feed - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination
- ❑ **B.A.M. (Bacteriological Analytical Manual)** – FDA's laboratory procedures for microbiological analyses of foods and cosmetics (previous editions by Association of Official Analytical Chemists)