Approaches for deriving microbiological criteria from performance objectives and performance criteria

Susanne Dahms

Relating Microbiological Testing and Microbiological Criteria to Public Health Goals
Washington DC, October 31 – November 1, 2005

Overview

- Microbiological criteria
- Sampling plans: Design and means to study their performance
- Food safety objectives
- Microbiological sampling plans and food safety objectives / performance objectives
  - Food safety / performance objective implicit in a given sampling plan
  - Development of sampling plan based on a prespecified food safety / performance objective

Microbiological Criteria - Purpose

A microbiological criterion defines the acceptability of a product or a food lot,
based on the absence or presence,
or number of microorganisms including parasites,
and/or quantity of their toxins/metabolites,
per unit(s) of mass, volume, area, or lot .

Microbiological Criteria - Definition

Requirements for a food to be considered safe are defined by stating:

- Microorganism representing the hazard and reasons for concern
- Analytical method to be used for detection and/or quantification
- Sampling plan to be applied in lot testing:
  - number of samples to be drawn
  - size of samples (analytical units)
  - microbiological limits
  - maximum allowed number of non-conforming samples (decision rule saying when to reject a lot)

Codex Alimentarius: CAC/GL 21-1997 (1977, 1996)

Microbiological Criteria - Example

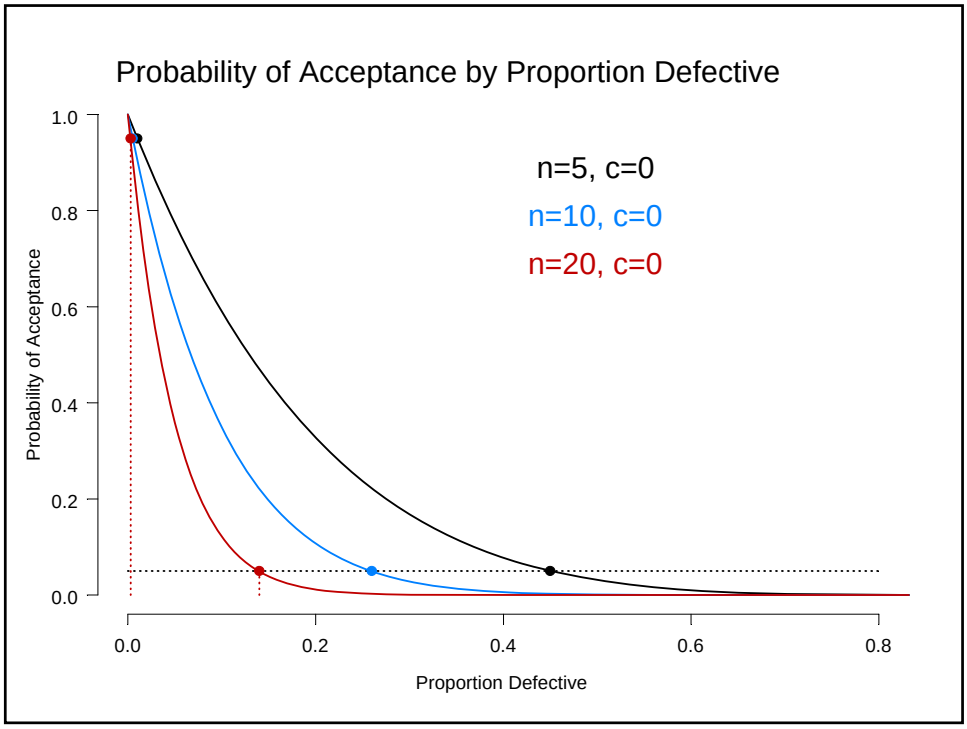
- *Listeria monocytogenes* in cold-smoked salmon
- Colony counting method
- Sampling plan:

  - 10 samples (analytical units) of 25g each
  - microbiological limit at 100 cfu/g
  - none of 10 samples is allowed to show an analytical result exceeding the microbiological limit of 100 cfu/g

Two-Class Attributes Sampling Plans

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

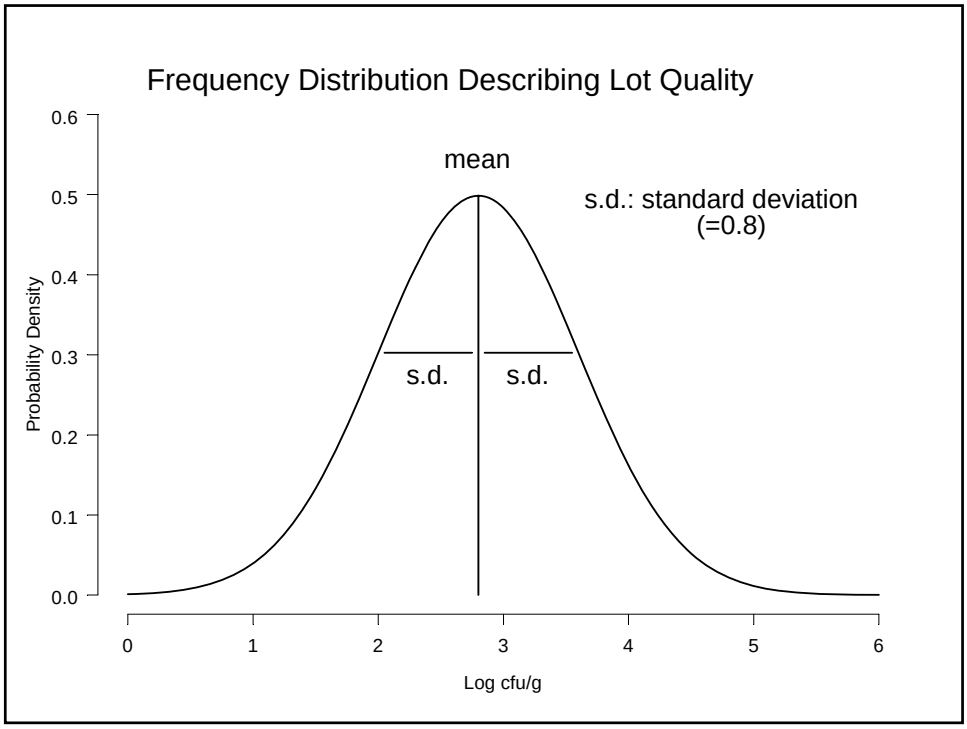
- n – number of sample units to be chosen independently and randomly from the lot
- m – a microbiological limit (i.e. in cfu/g); a sample is defined to be positive, if its microbial content exceeds this limit
- c – maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit m; for pathogens c is usually set to 0



OC Curve Referring to Mean Log cfu/g

Alternative approach for quantitative data:

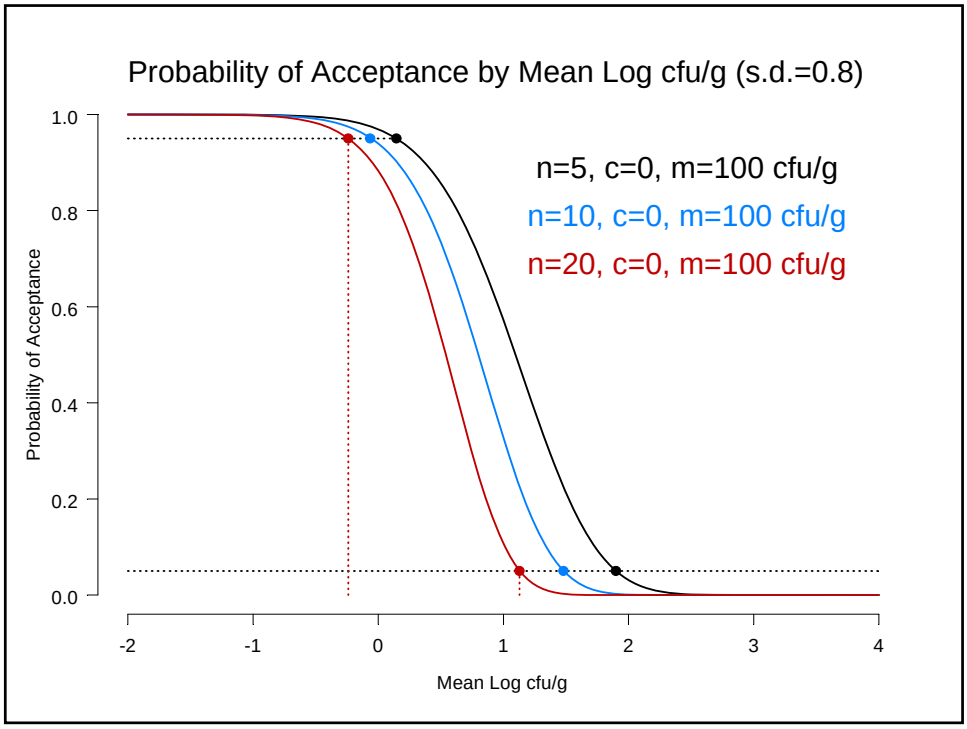
- Distributional assumption for sampling results
  e.g. log-normal with standard deviation known from previous experience

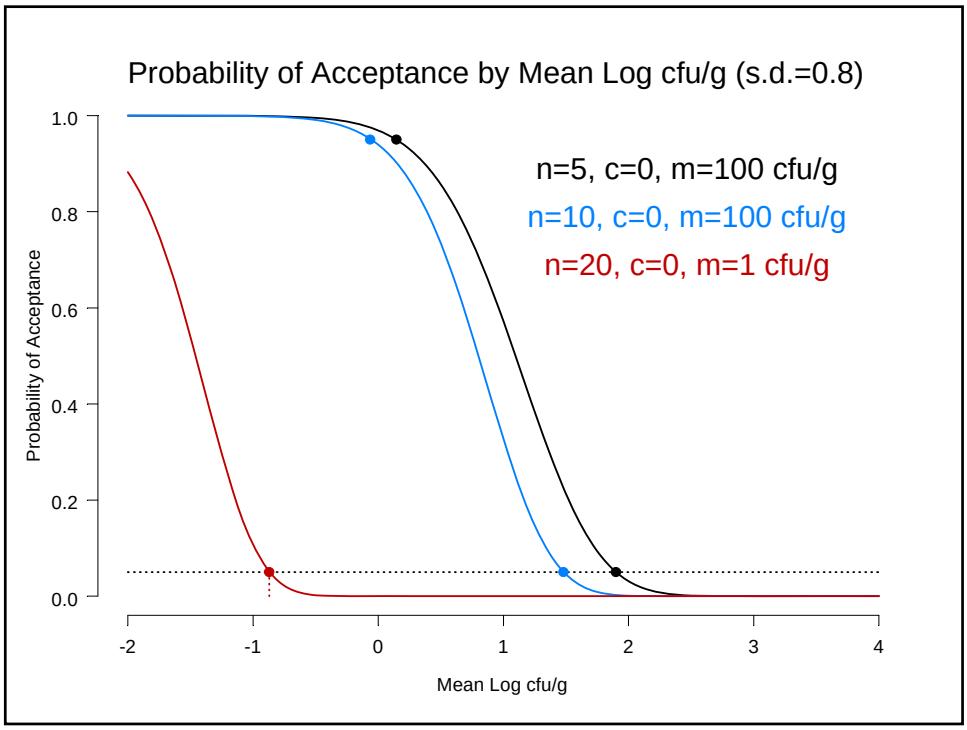


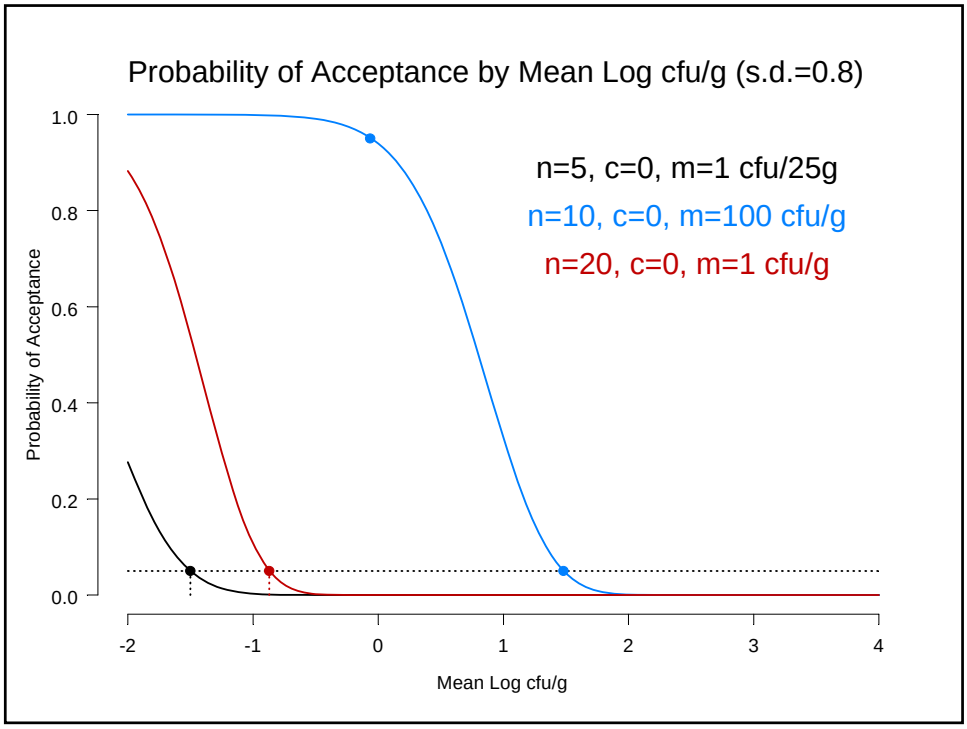
OC Curve Referring to Mean Log cfu/g

Alternative approach for quantitative data:

- Distributional assumption for sampling results
  e.g. log-normal with standard deviation known from previous experience
- Determine proportions acceptable and defective for possible mean log cfu/g
- Calculate acceptance probabilities and plot against mean log cfu/g


Probability of Acceptance by Mean Log cfu/g (s.d.=0.8)
n=5, c=0, m=100 cfu/g
n=10, c=0, m=100 cfu/g
n=20, c=0, m=100 cfu/g
Probability of Acceptance
1.0
0.8
0.6
0.4
0.2
0.0
-2
-1
0
1
2
3
4
Mean Log cfu/g


Probability of Acceptance by Mean Log cfu/g (s.d.=0.8)
n=5, c=0, m=100 cfu/g
n=10, c=0, m=100 cfu/g
n=20, c=0, m=1 cfu/g
Probability of Acceptance
1.0
0.8
0.6
0.4
0.2
0.0
-2
-1
0
1
2
3
4
Mean Log cfu/g



Performance of Sampling Plans

Sampling plan stringency, steepness of OC curve, location of critical lot qualities (95% probability of rejection, 95% probability of acceptance) depend on

- Plan specifications n and c
- Microbiological limits (m in 2-class plans)
- Standard deviation s.d.

Food Safety Objectives (FSO)

- Microbiological counterpart to maximum residue levels as defined in food chemistry
- Maximum concentration and/or frequency of a (microbiological) hazard in a food at the time of consumption that provides the appropriate level of protection
- Based on quantitative risk analyses relating concentrations or prevalences of pathogens in foods with disease risks

Example:
100 cfu of *listeria monocytogenes* per g in cold-smoked salmon at time of consumption

Performance Objectives or Performance Criteria (PO)

- Objectives for earlier points in the process
- Derived from food safety objectives taking into account growth or reduction of microorganisms during the process
- Example:
  - FSO (per 50g serving) = 5.0 log cfu/50g
  - FSO (per g) = 3.3 log cfu/g
  - Growth between point of sampling and point of consumption: 0.6 log cfu/g
  - PO = 2.7 log cfu/g

Statistical Interpretation of FSO / PO

Tentative approach:

FSO / PO as the ‚upper bound' of that frequency distribution of microbial concentrations that – if being tested - should be rejected with 95% probability.

‚Upper bound' could be defined as:

- 99%-quantile of the frequency distribution
- mean value + 3 x standard deviation

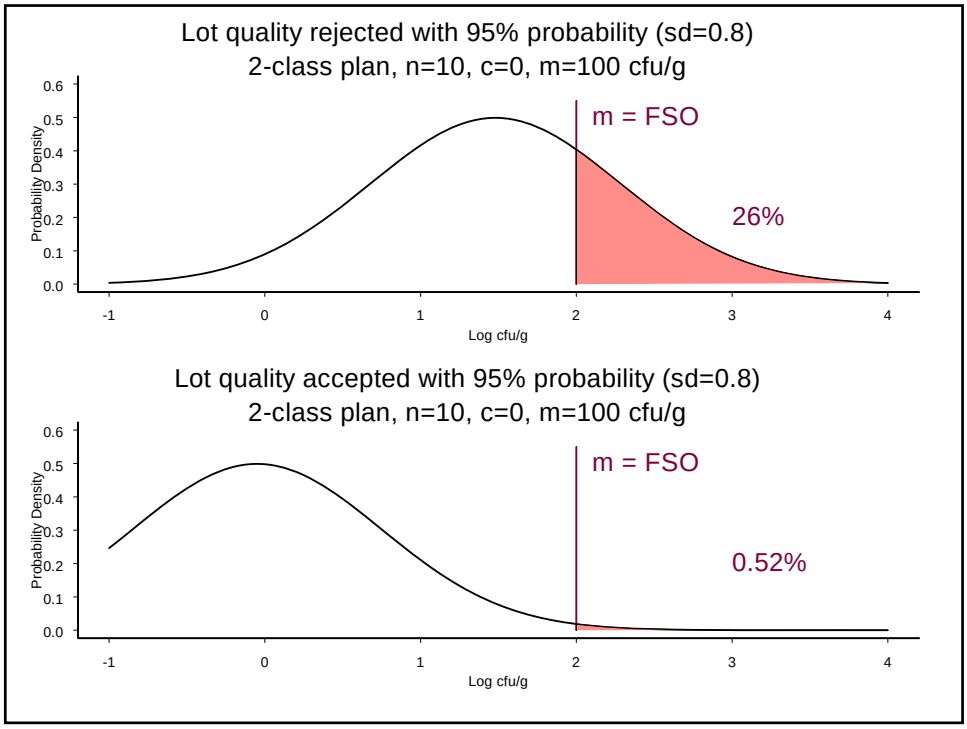
Sampling plans and FSOs: Example *Listeria Monocytogenes*

Proposed sampling plan:
no inactivation, growth not assumed to occur
n = 10 sample units with c = 0 and m = 100 cfu/g

ICMSF (1994) Int. J. Food Microbiol. 22:89-96
CODEX ALIMENTARIUS COMMISSION, August 2001, CX/FH 01/6 ANNEX 3.2

Assuming a standard deviation of s.d. = 0.8 log units
- mean contamination rejected with 95% probability:
  1.48 log cfu/g
- mean contamination accepted with 95% probability:
  -0.05 log cfu/g

(corresponding to 30 cfu/g and 1 cfu/g)



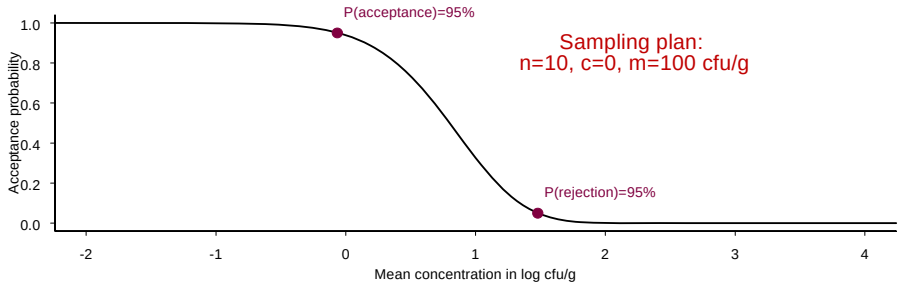
Food safety / performance objective implicit in a given sampling plan

Assuming

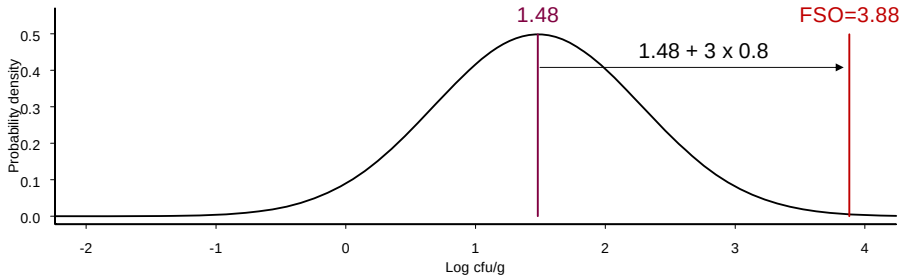
FSO (or PO) = mean value + 3 x standard deviation

the implicit FSO (or PO) can be derived from the sampling plan operation characteristics

1. Probability of acceptance by mean concentration in log cfu/g (sd = 0.8)



2. Lot rejected with 95% probability (sd = 0.8)



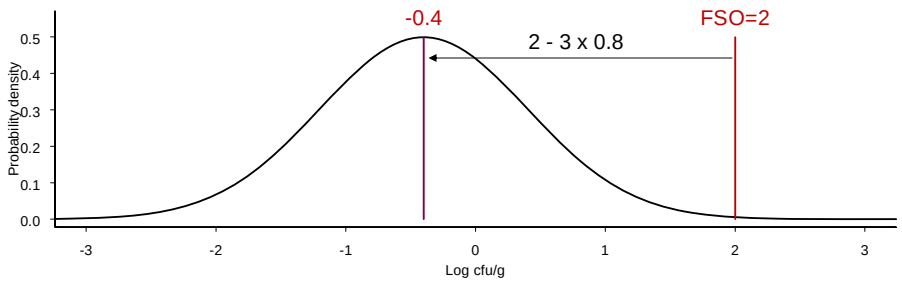
Sampling plan based on a prespecified food safety / performance objective

Using the relationship the other way round:

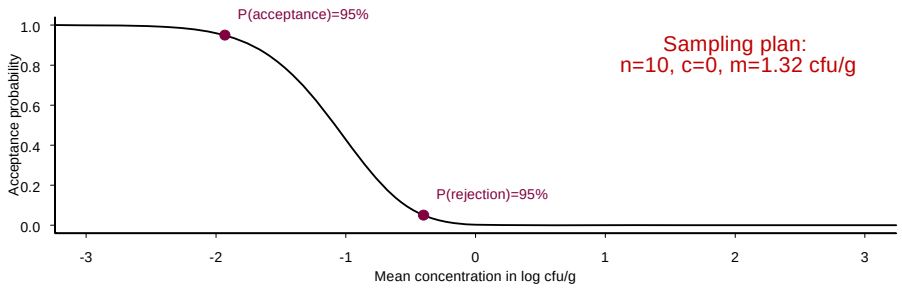
mean value = FSO (or PO) - 3 x standard deviation

for a prespecified FSO (or PO) that mean concentration level can be determined that should be rejected with 95% probability when a sampling plan is applied

1. Lot rejected with 95% probability (sd = 0.8)

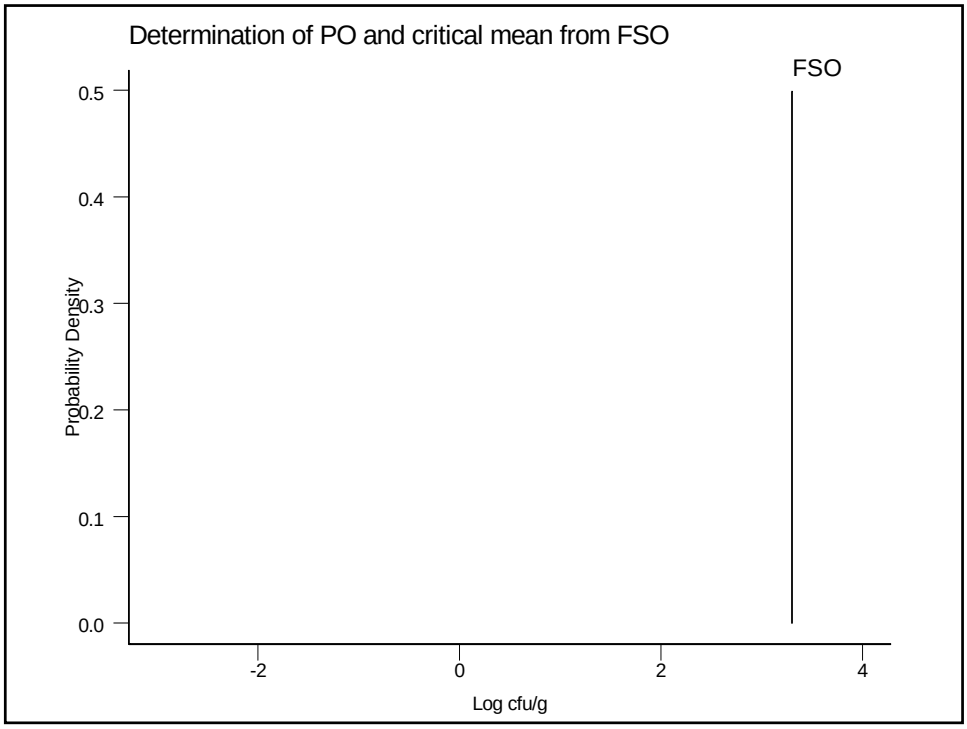


2. Probability of acceptance by mean concentration in log cfu/g (sd = 0.8)



Development of Sampling Plans Based on Specified FSO (or PO)

1. Based on a given FSO per serving the FSO value per g is derived

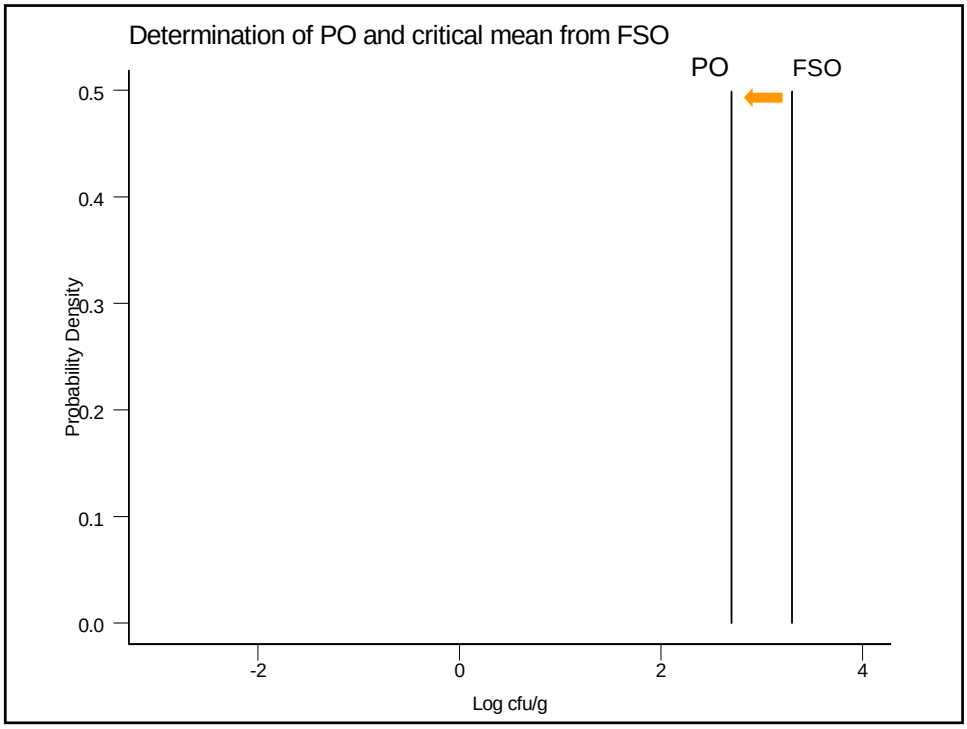


Development of Sampling Plans Based on Specified FSO

2. The process between point of consumption and point of sampling has to be analysed with regard to growth or reduction of microorganisms and resulting concentrations in the respective food

3. Based on these considerations the performance objective (PO) at point of sampling is determined as:

PO = FSO ± growth / reduction



Development of Sampling Plans Based on Specified FSO

The PO is interpreted as:

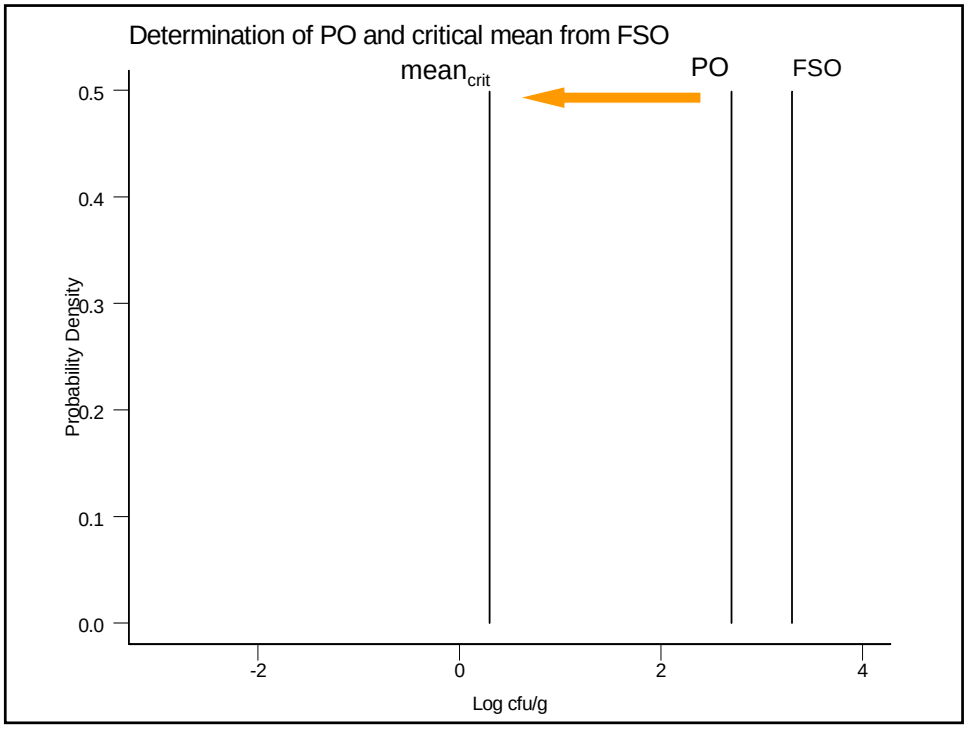
$$PO = mean_{crit} + 3 \times \text{standard deviation}$$

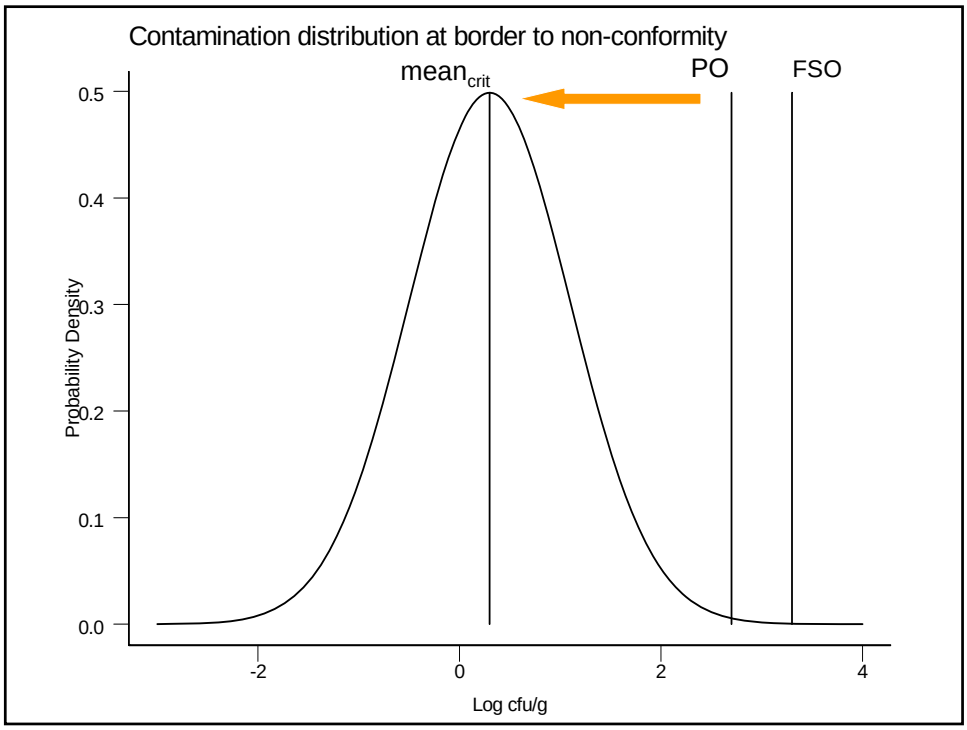
$mean_{crit}$ is the mean (in log cfu/g) of the maximally acceptable concentration distribution,
the assumed standard deviation should be based on previous experience

Therefore

4. The maximally acceptable mean (in log cfu/g) is determined as:

$$mean_{crit} = PO - 3 \times \text{standard deviation}$$


Determination of PO and critical mean from FSO
mean$_{crit}$
PO
FSO
Probability Density
0.0
0.1
0.2
0.3
0.4
0.5
-2
0
2
4
Log cfu/g


Contamination distribution at border to non-conformity
mean$_{crit}$
PO
FSO
Probability Density
0.0
0.1
0.2
0.3
0.4
0.5
-2
0
2
4
Log cfu/g

Development of Sampling Plans Based on Specified FSO

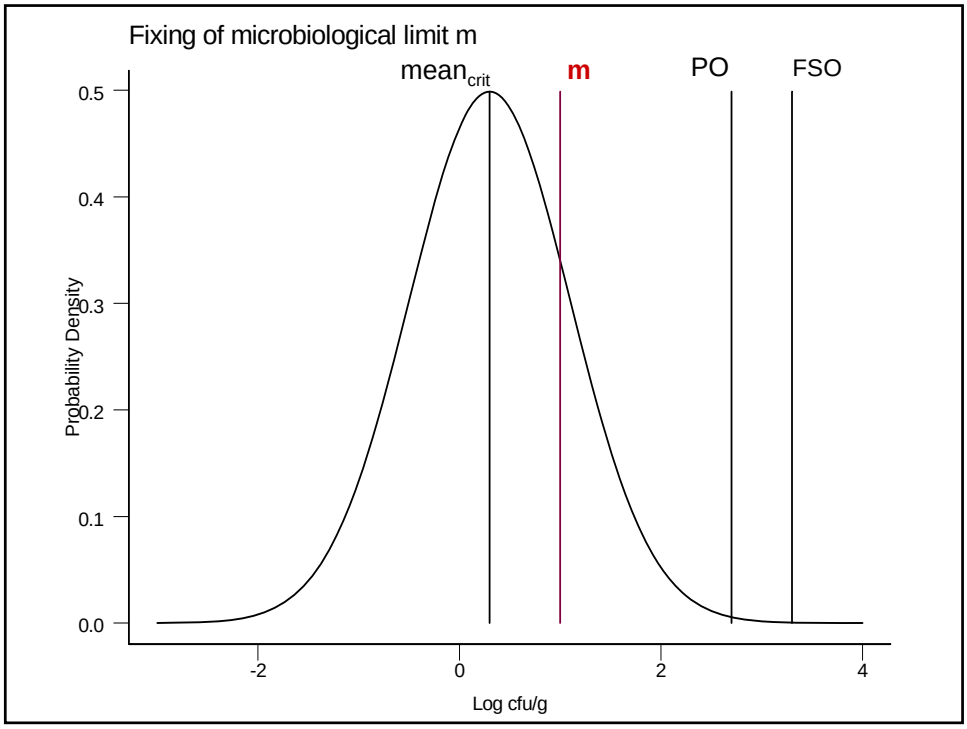
5. The required probability of rejecting a non-conforming lot has to be specified, denoted as:

$$1 - \alpha$$

Non-conforming corresponds to a mean value that exceeds $mean_{crit}$

Development of Sampling Plans Based on Specified FSO

6. It has to be decided which analytical method to use on the samples. The choice of a suitable value for the microbiological limit m depends on this decision.
   - For presence/absence testing in 25g samples m would be
     1/25 = 0.04 cfu/g on the original scale or
     -1.39 log cfu/g on the logarithmic scale (base 10)
   - Using the quantitative plating method 100 cfu/g on the original scale or 2 log cfu/g on log scale could be taken as m
     (for instance for *L. monocytogenes*)

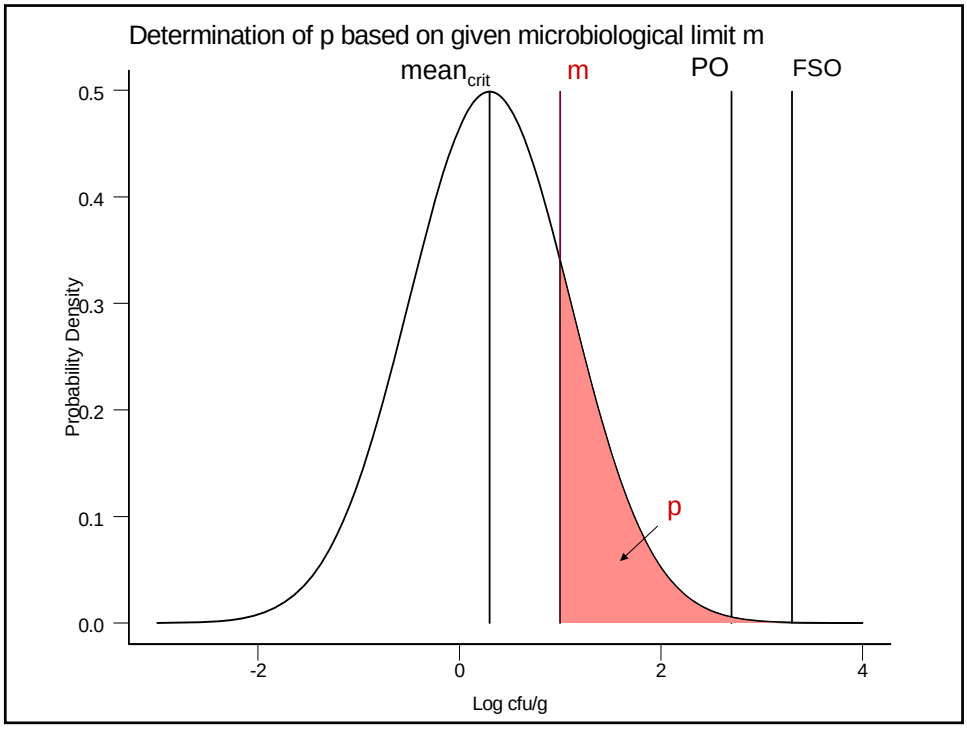


Development of Sampling Plans Based on Specified FSO

7. Calculation of the number of samples, n, providing the desired probability of rejecting non-conforming lots is then done in two steps:

   First:

   For chosen m the probability p that a single sample will exceed the microbiological limit m is calculated for a lot with mean$_{crit}$.



Development of Sampling Plans Based on Specified FSO

7. Calculation of n

Second:

Assuming that c should be 0 for the sampling plan, based on p the number of samples is derived that is required to find at least one unit exceeding limit m with given probability for rejection:

Prob(no of 'positive' samples ≥ 1) = 1 - α

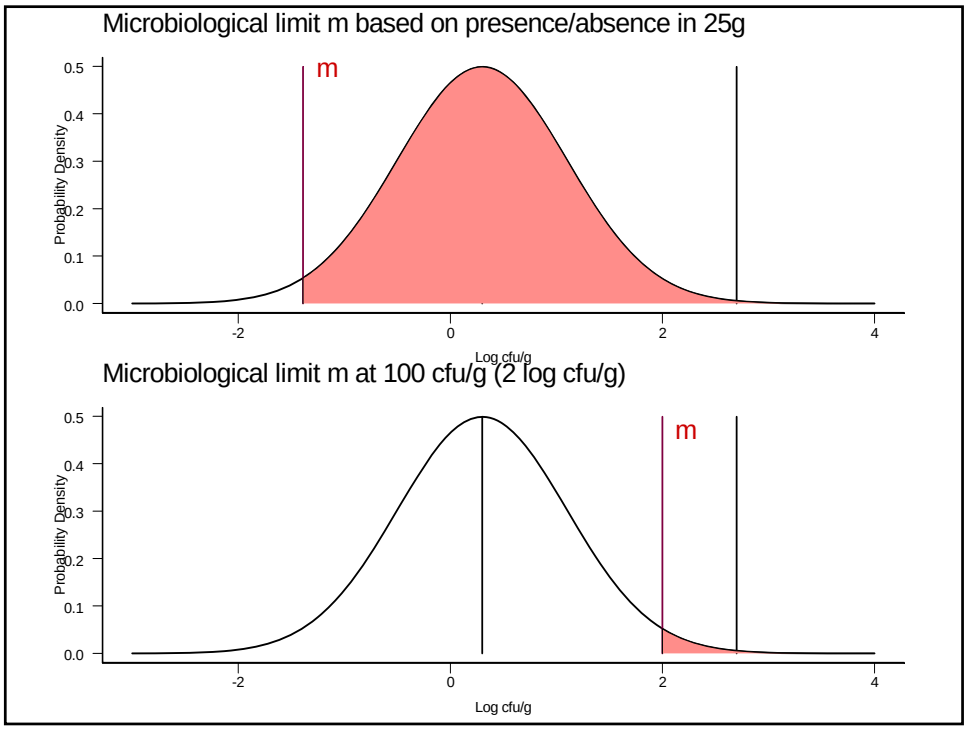
Based on a binomial distribution this leads to:

$n \geq \log \alpha / \log (1-p)$

Number of Samples - Example

FSO (per 50g serving) = 5.0 log cfu/g
FSO (per g) = 3.3 log cfu/g
PO (per g) = 2.7 log cfu/g

m	sd = 0.4 $mean_{crit}$ = 1.5	**sd = 0.8** **$mean_{crit}$ = 0.3**
0.04 cfu/g **(-1.39 log cfu/g)**	1	**1**
100 cfu/g **(2 log cfu/g)**	27	**177**
10 cfu/g (1 log cfu/g)	2	**15**



Relation $mean_{crit}$ to m and Number of Samples

m =	n =
$mean_{crit}$	5
$mean_{crit}$ + 0.5 x standard deviation	9
$mean_{crit}$ + 1 x standard deviation	18
$mean_{crit}$ + 2 x standard deviation	131
$mean_{crit}$ + 3 x standard deviation = PO	2218

Conclusion

To derive microbiological criteria from performance objectives a firm understanding of sampling plans and their statistical background is required.

To find efficient attributes sampling plans the choice of microbiological limits in relation to critical mean concentration levels is a crucial point.

The choice of suitable microbiological limits depends on feasible analytical techniques available for that purpose

→ it‘s about quantification!

Acknowledgements:

Members of ICMSF, especially Paul Teufel