FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

Statistics underlying Sampling Plans for Microbiological Criteria

Delivered by:

Marcel Zwietering

WAGENINGEN UNIVERSITY & RESEARCH

Sampling plan: example

Food category: powdered infant formulae (PIF)

Safety Criteria:

Microorganism	Sampling plan		Sample weight (g)	Analytical method
	n	c		
Cronobacter spp.	30	0	10	ISO/TS 22964
Salmonella	60	0	25	ISO 6579

CODEX Code of hygienic practice for powdered formulae for infants and young children CAC/RCP 66-2008

Qualitative, 2 class, c=0

Sampling plan: example

Food category: powdered infant formulae (PIF)

Hygiene Criteria:

Micro-organism	Sampling plan		m	M	Analytical method
	n	c			
Mesophiles	5	2	500/g	5000/g	ISO 4833
Enterobacteriaceae	10	2	0/10 g	-	ISO 21528-1/21528-2

CODEX Code of hygienic practice for powdered formulae for infants and young children CAC/RCP 66-2008

Quantitative, 3 class, c≠0

Qualitative, 2 class, c≠0

The anatomy of a sampling plan

Qualitative and Quantitative plans:

+/-: 0/25g 0/10g ≤100 cfu/g or >100 cfu/g

2 class and 3 class plans

2: +/- ≤100 cfu/g / >100 cfu/g 3: x≤500 /g; 500<x≤5000; >5000/g

c= 0 or *c*≠0

Class	Qual/Quant	c=0 ?
2	Qual	0
2	Qual	≠
2	Quan	0
2	Quan	≠
3	Quan	≠

The anatomy of a sampling plan

Microorganism	Sampling plan		Sample weight (g)	Analytical method
	n	c		
Cronobacter spp.	30	0	10	ISO/TS 22964
Salmonella	60	0	25	ISO 6579

2-class, qualitative, c=0

Cronobacter PIF (2-class, qualitative)

n=30 *c*=0 *m*=0/10g

30 samples

None of 30 samples is allowed to show an analytical result exceeding the microbiological limit

Microbiological limit (defective at 1 cfu/10 g or more)

Sampling plan: example

Micro-organism	Sampling plan		m	M	Analytical method
	n	c			
Mesophiles	5	2	500/g	5000/g	ISO 4833
Enterobacteriaceae	10	2	0/10 g	-	ISO 21528-1/21528-2

2-class, qualitative, c=2

Enterobacteriaceae PIF (2-class, qualitative)

n=10 *c*=2 *m*=0/10 g

10 samples

Two of 10 samples are allowed to show an analytical result exceeding the microbiological limit

Microbiological limit (defective *sample* at 1 cfu/10 g or more)

Sampling plan: example

Micro-organism	Sampling plan		m	M	Analytical method
	n	c			
Mesophiles	5	2	500/g	5000/g	ISO 4833
Enterobacteriaceae	10	2	0/10 g	-	ISO 21528-1/21528-2

3-class, quantitative, c=2

Mesophiles – PIF (3-class, quantitative)

n=5 c_m=2 m=500/g M=5000/g

5 samples

Two of 5 samples are allowed to show an analytical result exceeding the microbiological limit m but not M

Microbiological limit (marginal defective) at >500 cfu/g

Microbiological limit (defective) at >5,000 cfu/g

Sampling plan:

Ready-to-eat (no growth) foods from the end of manufacture or port of entry (for imported products), to the point of sale

Micro-organism	Sampling plan		m	M	Analytical method
	n	c			
Listeria monocytogenes	5	0	100 cfu/g	-	ISO 11290-2

2-class, quantitative, c=0

Listeria– no growth (2-class, quantitative)

n=5 *c*=0 *m*=100 /g

5 samples

None of the 5 samples are allowed to show an analytical result exceeding the microbiological limit *m*

Microbiological limit (defective) at >100 cfu/g

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Gover

CHIFSS
CII-HUL Initiative on Food Safety Sciences

ANNEX

Annex I to Regulation (EC) No 2073/2005 is amended as follows:

(1) in Chapter 2, Section 2.1 is amended as follows:

(a) the table is amended as follows:

(ii) the following row 2.1.9 is added:

Food category	Micro-organisms	Sampling plan		Limits		Analytical reference method	Stage where the criterion applies	Action in case of unsatisfactory results
		n	c	m	M			
"2.1.9 Carcases of broilers	*Campylobacter* spp.	50 ([5])	c=20 From 1.1.2020 c=15; From 1.1.2025 c=10	1000 cfu/g		EN ISO 10272-2	Carcases after chilling	Improvements in slaughter hygiene, review of process controls, of animals origin and of the biosecurity measures in the farms of origin

2-class, quantitative, c=20..15..10

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

Sampling plan:

Hygiene criterion *Campylobacter* broilers

Micro-organism	Sampling plan		m	M	Analytical method
	n	c			
Campylobacter spp.	50	20	1000 cfu/g	-	ISO 10272-2

2-class, quantitative, c=20..15..10

Campylobacter–broilers (2-class,quantitative)

n=50 *c*=20 *m*=1000 /g

50 samples

20 of the 50 samples are allowed to show an analytical result exceeding the microbiological limit *m*

Microbiological limit (defective) at >1000 cfu/g

The anatomy of a sampling plan

Class	Qual/Quant	c=0 ?	Example
2	Qual	0	*Salmonella* in PIF
2	Qual	≠	*Enterobacteriaceae* in PIF
2	Quan	0	*Listeria* in no growth RTE
2	Quan	≠	*Campylobacter* in broilers
3	Quan	≠	Mesophiles in PIF

Verification by MicroCrit

Monitor Critical Limits

Validated CCPs

HACCP

PRP (GMP, GHP, ...)

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

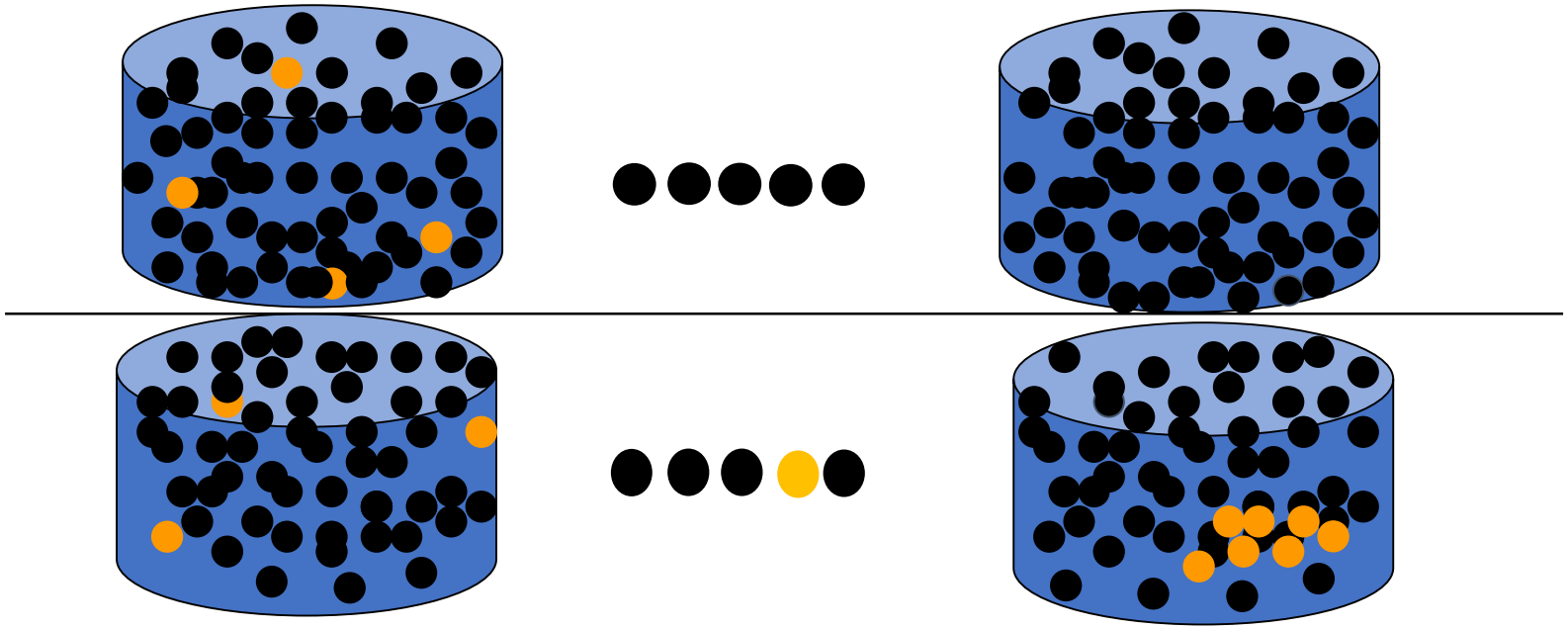
Ministry of Health and Family Welfare, Government of India

CHIFSS
CII-HUL Initiative on Food Safety Sciences

verification by MicroCrit

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

End product testing useful or lottery ?



Positives mean something, negatives are no guarantee

MISCONCEPTION 1

If the tested sample units are negative, the batch is free of the pathogen.

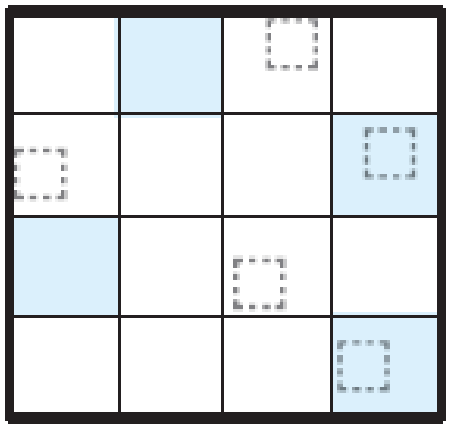
FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

Statistical Aspects of Food Safety Sampling

I. Jongenburger, H.M.W. den Besten, and M.H. Zwietering

Annu. Rev. Food Sci. Technol. 2015. 6:479–503

a



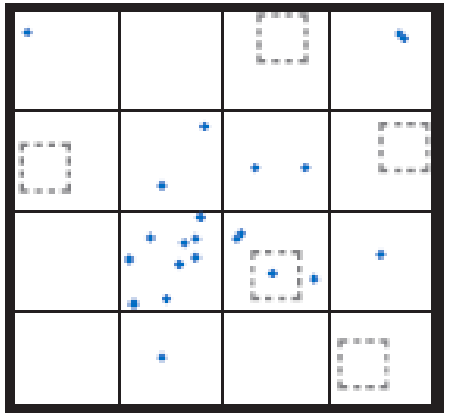
Homogeneous contamination

b

22	113	94	49
8	10	93	105
520	59	81	17
19	101	36	33

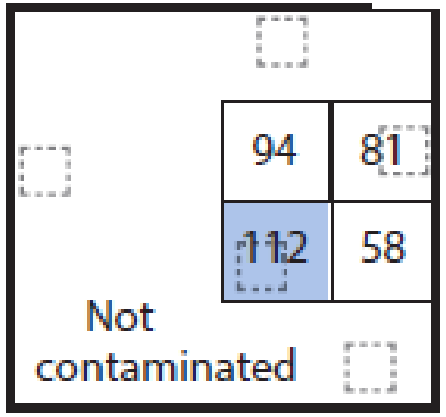
Heterogeneous high-level contamination

c



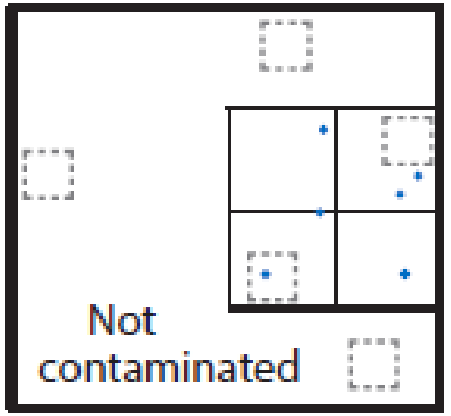
Heterogeneous low-level contamination

d



Localized high-level contamination

e

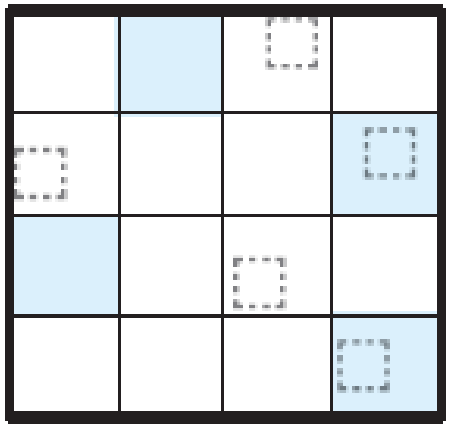


Localized low-level contamination

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

CHIFSS
CII-HUL Initiative on Food Safety Sciences

a



Homogeneous contamination

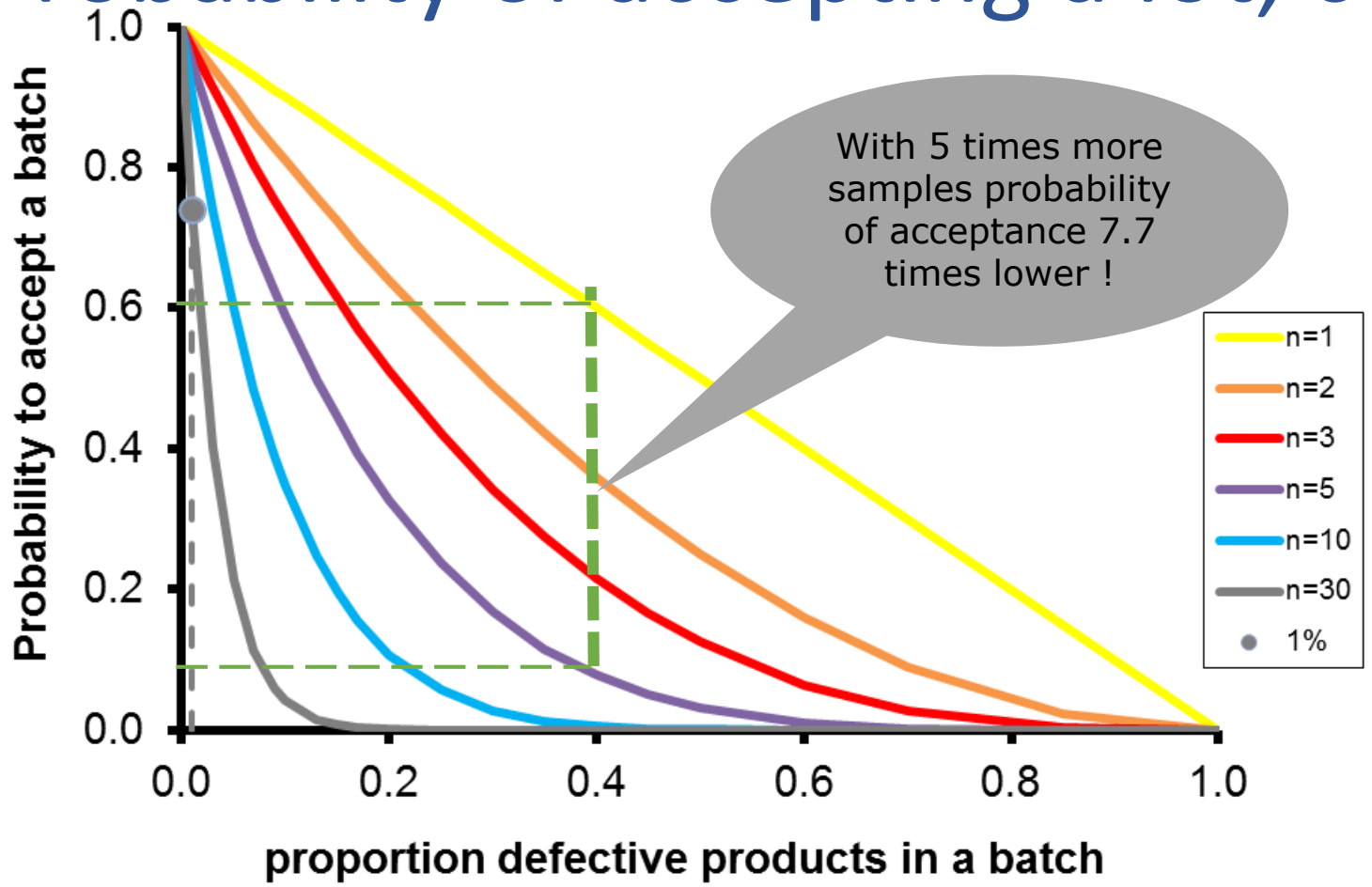
Probability that no contamination is found

$P_{defective}$	n=1
	1- P_{def} =
0.00	1.00
0.01	0.99
0.05	0.95
0.10	0.90
0.15	0.85
0.20	0.80
0.25	0.75
0.30	0.70

1 % defectives of 100,000 products, means 1,000 products

$$P_{accept} = (1 - P_{defective})^n$$

Probability of accepting a lot, c=0



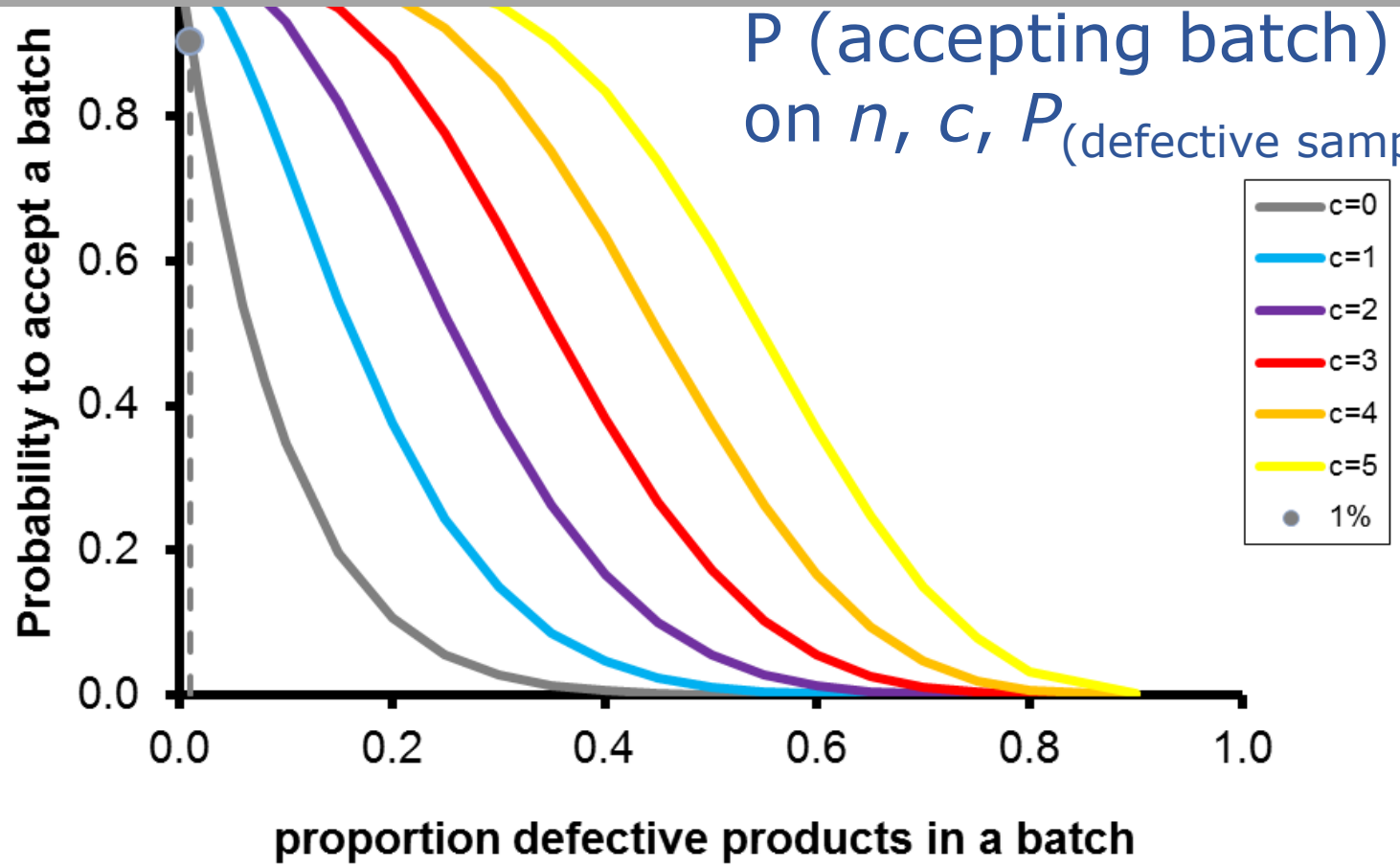
MISCONCEPTION 2

Using a realistic sampling scheme, it is possible to test for absence of a pathogen in a batch of food.

MISCONCEPTION 3

Current sampling plans assume that microorganisms follow the binomial distribution.

P (accepting batch): depends on n, c, $P_{(\text{defective sample})}$



$$If\ c \neq 0\ P_{accept} = binomial(k \leq c, n, P_{defective})$$

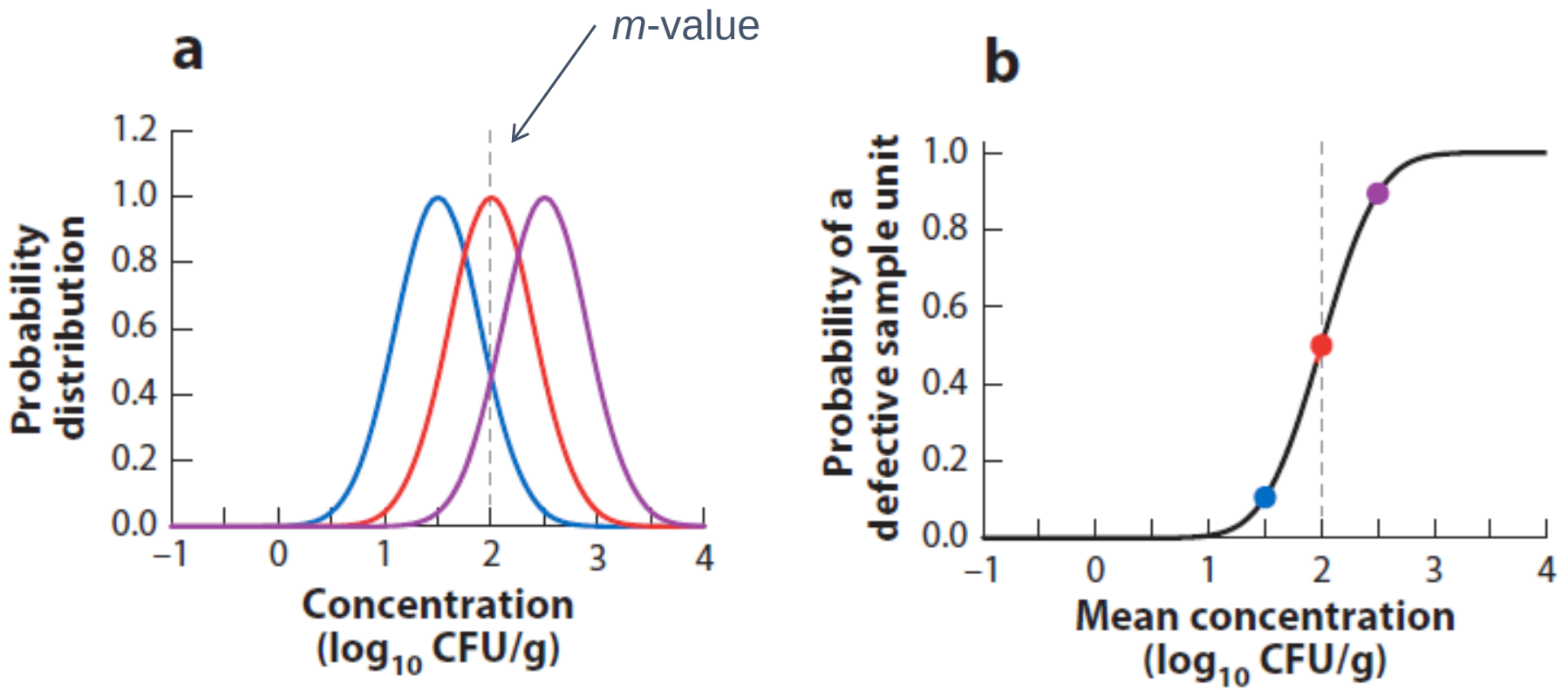
b

22	113	94	49
8	10	93	105
520	59	81	17
19	101	36	33

Heterogeneous high-level contamination

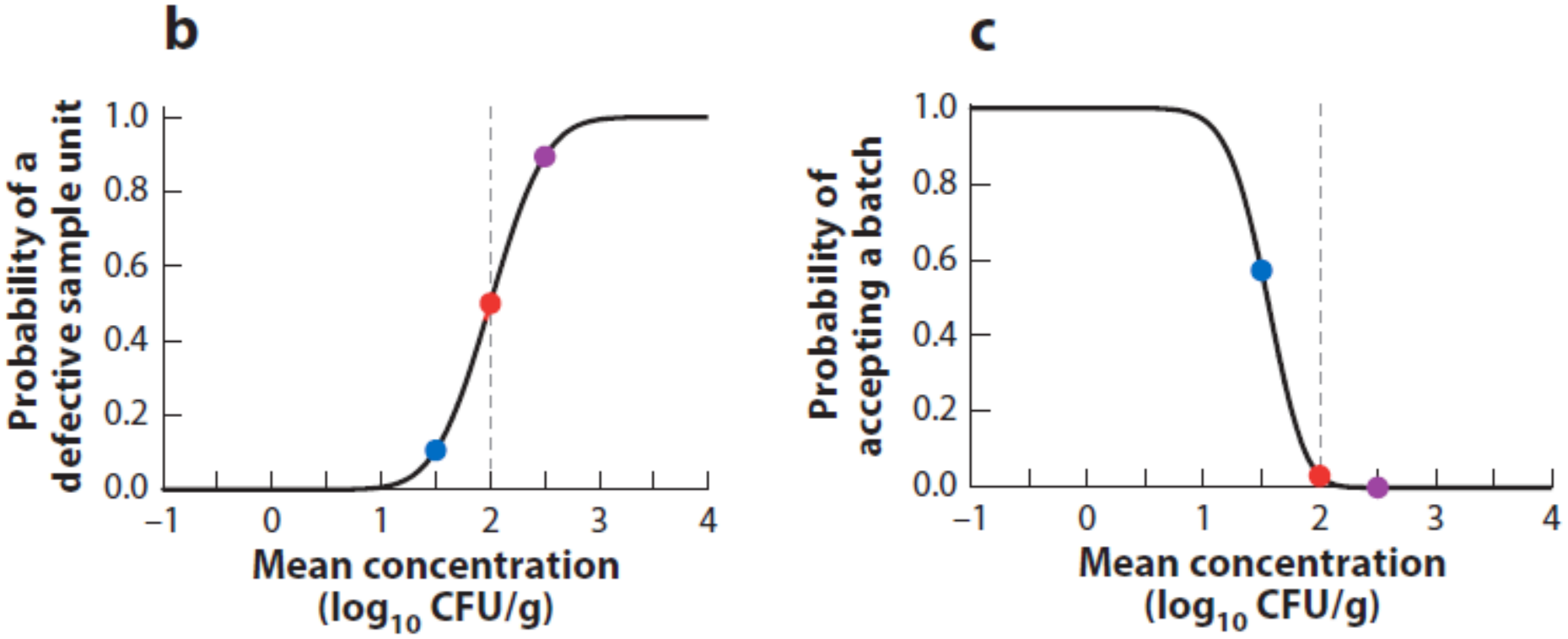
MISCONCEPTION 4

Current sampling plans assume that microorganisms are homogeneously distributed in a batch.



$$P_{defective} = P_{normal}(\log_{10} C > m, \mu_{\log C}, \sigma_{\log C})$$
$$= 1 - P_{normal}(\log_{10} C \leq m, \mu_{\log C}, \sigma_{\log C}),$$

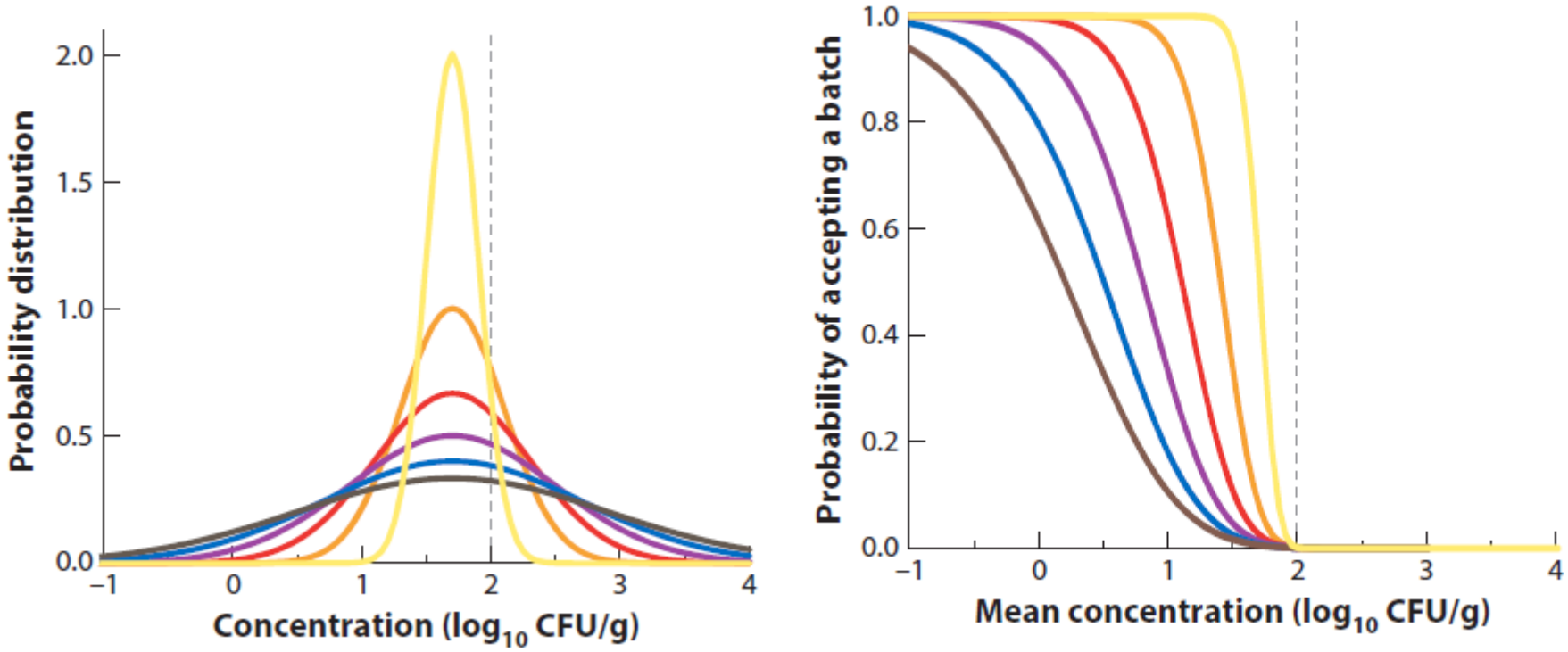
OC curve: Operating Characteristic



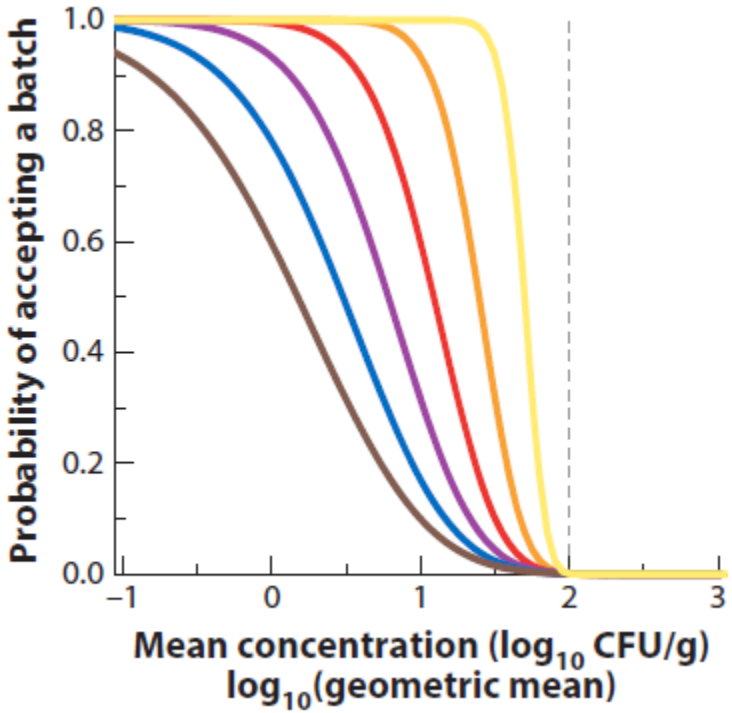
$$P_{accept}(c, n, P_{defective}) = binomial(k \leq c, n = n, P = P_{defective})$$

n=5

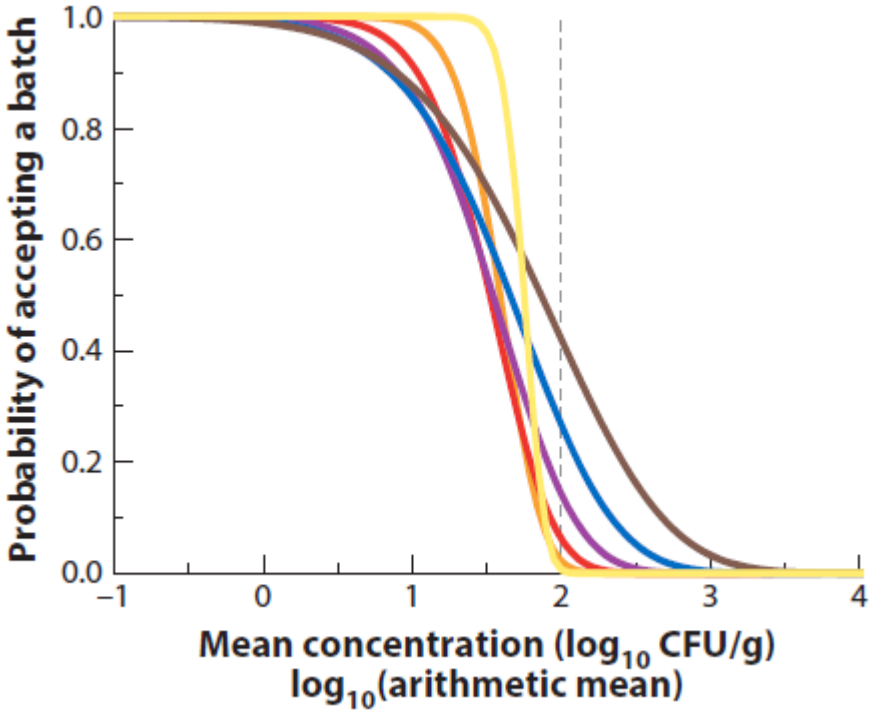
n=10; σ=1.2 (*brown),* 1.0 (*blue*), 0.8 (*purple*), 0.6 (*red*), 0.4 (*orange*), and 0.2 (*yellow*) $\log_{10}$ CFU/g.



n=10; σ=1.2 (*brown),* 1.0 (*blue*), 0.8 (*purple*), 0.6 (*red*), 0.4 (*orange*), and 0.2 (*yellow*) $\log_{10}$ CFU/g.



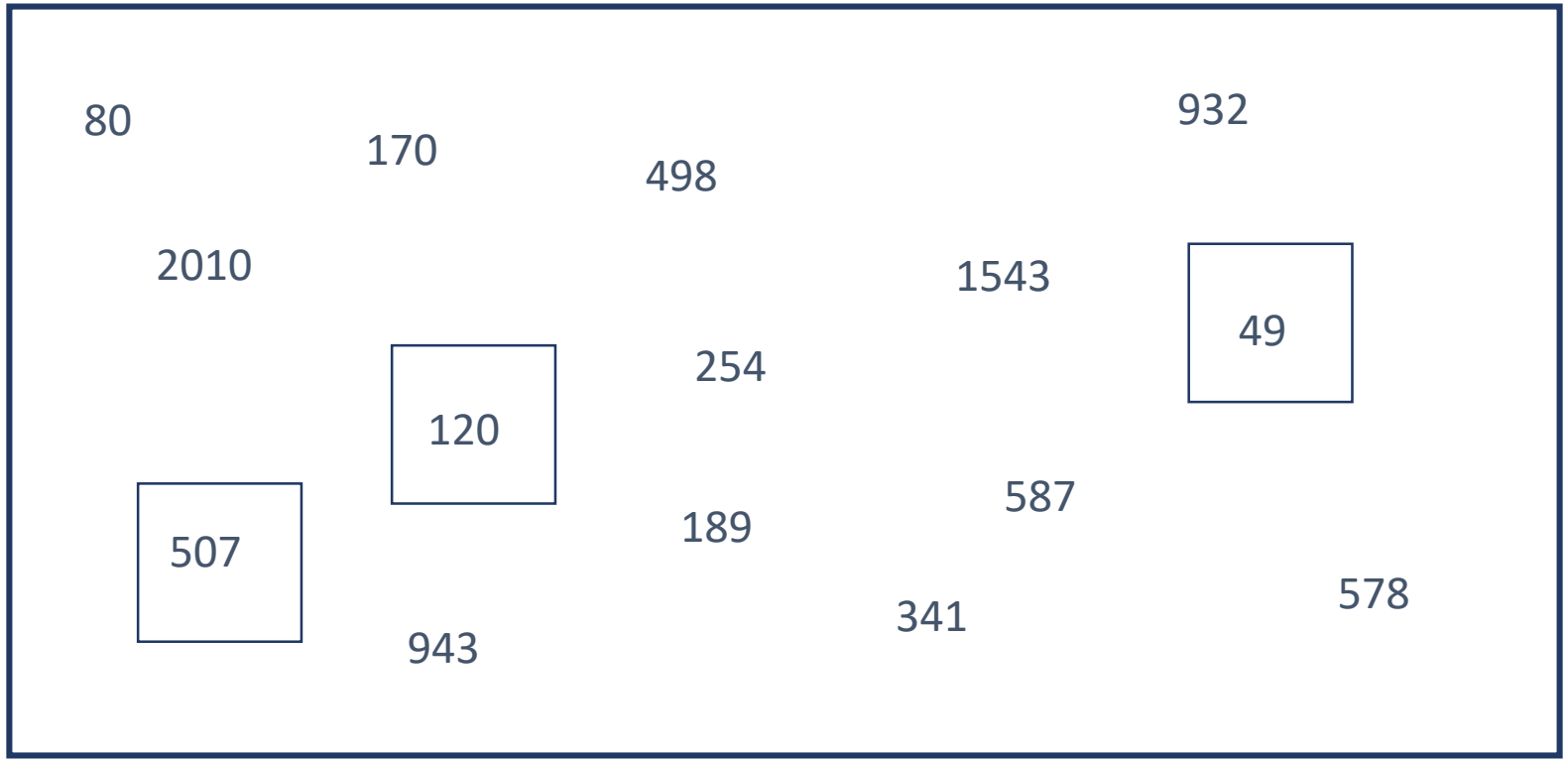
mean log



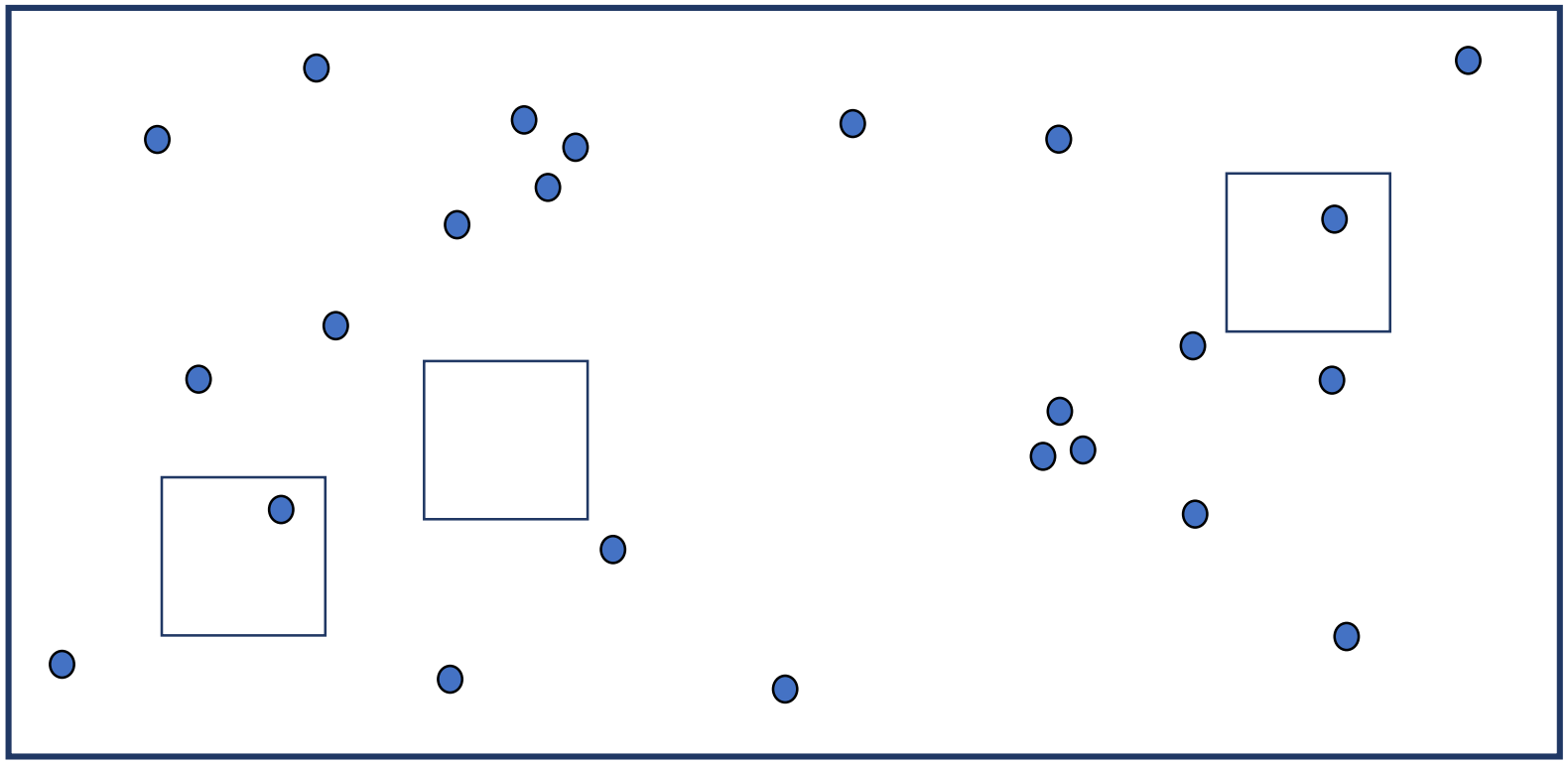
log mean

$$\log_{10}(\bar{C}) = \overline{\log_{10} C} + 0.5 \cdot \ln 10 \cdot \sigma^2_{\log_{10} C}$$

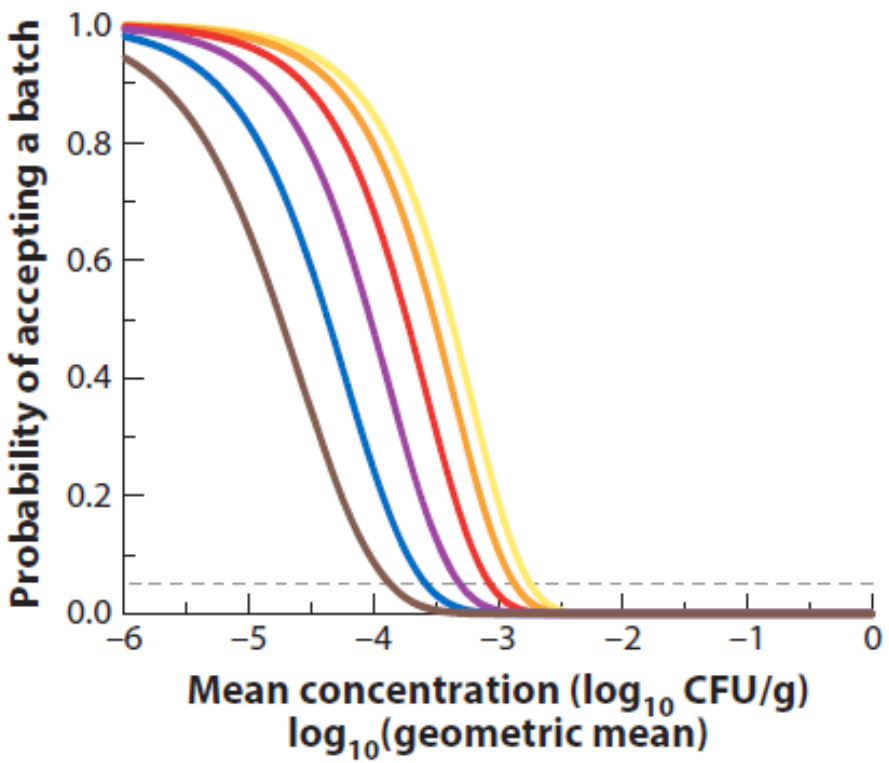
Distribution counts



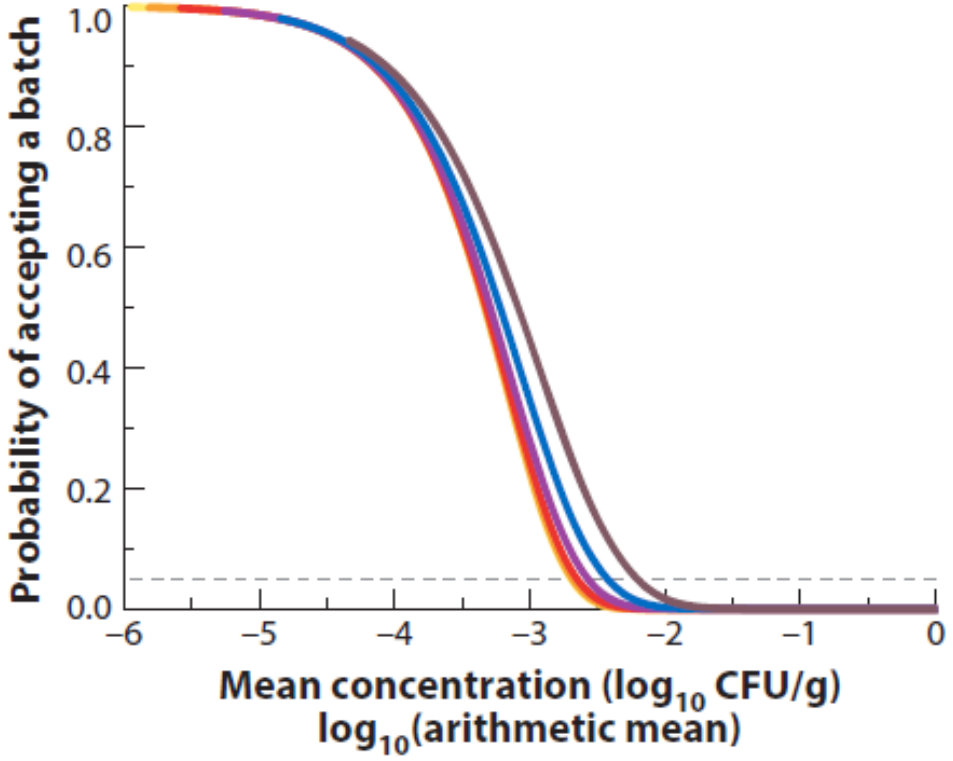
Distribution enrichment



n=60; σ=1.2 (*brown),* 1.0 (*blue*), 0.8 (*purple*), 0.6 (*red*), 0.4 (*orange*), and 0.2 (*yellow*) $\log_{10}$ CFU/g.



mean log



log mean

Three statistical phenomena are relevant:

1. the actual spatial distribution of microorganism in the food batch,
2. the statistical process of taking a sample unit and it being defective
3. the acceptance of the lot based on *n* sample units, of which *c* are accepted to be positive and $P_{defective}$

For example

1. organism lognormally distributed in product
2. taking one sample is a Poisson process

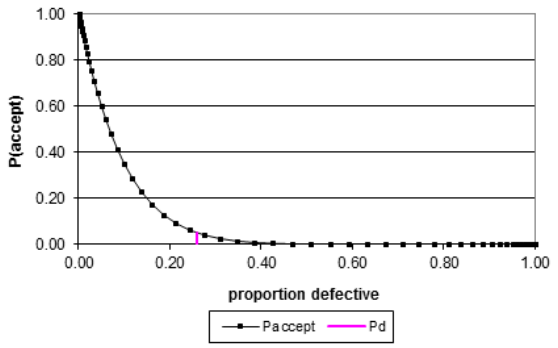
   $P_{\text{defective}}$ is a Poisson-lognormal distribution of contaminant in the sample unit
3. P_{accept} of a lot based on $P_{defective}$, *n* sample units, and *c* is a binomial process

   P_{accept} is then a Binomial(Poisson(LogNormal)) distribution !

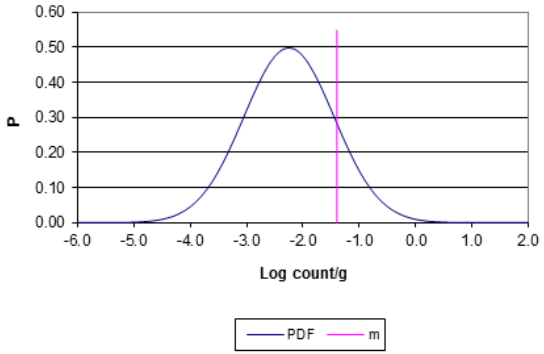
FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

http://www.icmsf.org

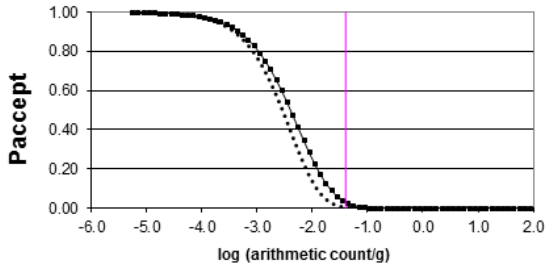
Operating characteristic curve for proportion defective, with n=10 and c=0



Probability density function (PDF) for log counts. Distribution mean = -2.25 and sigma = 0.80



Operating characteristic curve scaled to relate log arithmetic mean count to m



Batch acceptance for Pd		P(accept)
Pd	20 %	10.7 %
actualPd	25.9 %	5.00 %

INPUTS		P(accept)	
mean	-2.25	Computed	5.00 %
sigma	0.80	Desired	5 %
m	-1.40		
n	10		
c	0		
amount	25 g		

Find mean that gives desired P(accept)

Find n that gives desired P(accept) or better (less)

Preject 95.00

ALTERNATIVE n AND c		P(accept)	
mean	-2.25	Computed	0.91 %
sigma	0.80	Target, left	5.00 %
m	-0.98		
n	30		
c	0		
amount	9.6 g		

For any value of n and c imputed find the m that gives the same P(accept) as the model on the left

Sandbox: for your own calculations

Means and median

	Arithmetic			Geometric=median	
	0.0307	cfu/g		0.0056	cfu/g
one cfu in	32.6	grams	one cfu in	177.7	grams
	-1.51	log cfu/g		-2.25	log cfu/g

Implied Acceptance level

Percentile	z-score	Concentration at this percentile
99.9	3.10	0.23

This sampling plan would provide 95 % confidence that a lot of food containing a median concentration of 1 organism in 177.7 g and an average concentration of 1 organism in 32.6 g (and having a standard deviation of 0.80 log cfu/g), would be rejected (i.e. more than 0 out of 10 samples of 25 grams giving detection of the organism)

About | Technical issues | Introduction | **2class enrichment** | 2class counts | 3class counts

Conclusions

- All samples negative is no guarantee of safety
- A positive sample is indicating unsafety
- Sampling is useful for verification
- As function of the arithmetic mean the effect of the spread is limited
- Tools exist !

Control of safety is only to a very limited extend supported by end-product testing