

Principles of microbiological testing: Statistical basis of sampling



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Symposium on Relating Microbiological Testing and Microbiological Criteria to Public Health Goals

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Overview

- Sampling plans
- ICMSF Cases
- Statistical basis
- Performance of sampling plans
- Summary and Conclusions

Sampling Plans

- Define the probability of detecting a microorganisms or other hazards in a lot
- None can ensure the absence of a particular hazard
- Should be administratively and economically feasible

Types of Microbiological Sampling Plans

Attributes 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)

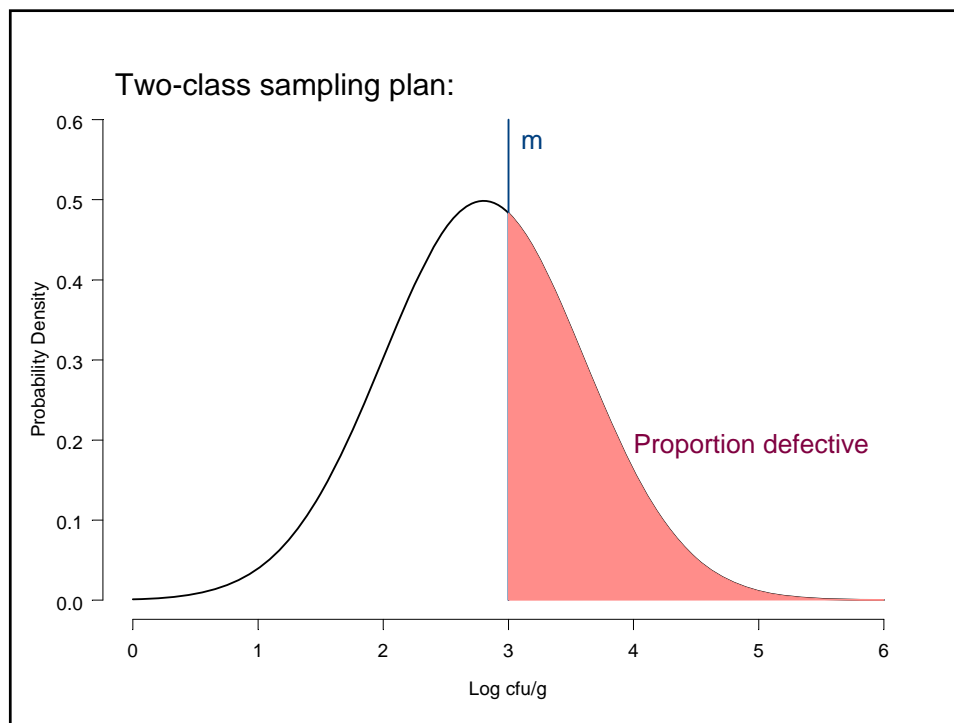
Variables plans:

Non-grouped quantitative analytical results
Require distributional assumptions be made

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



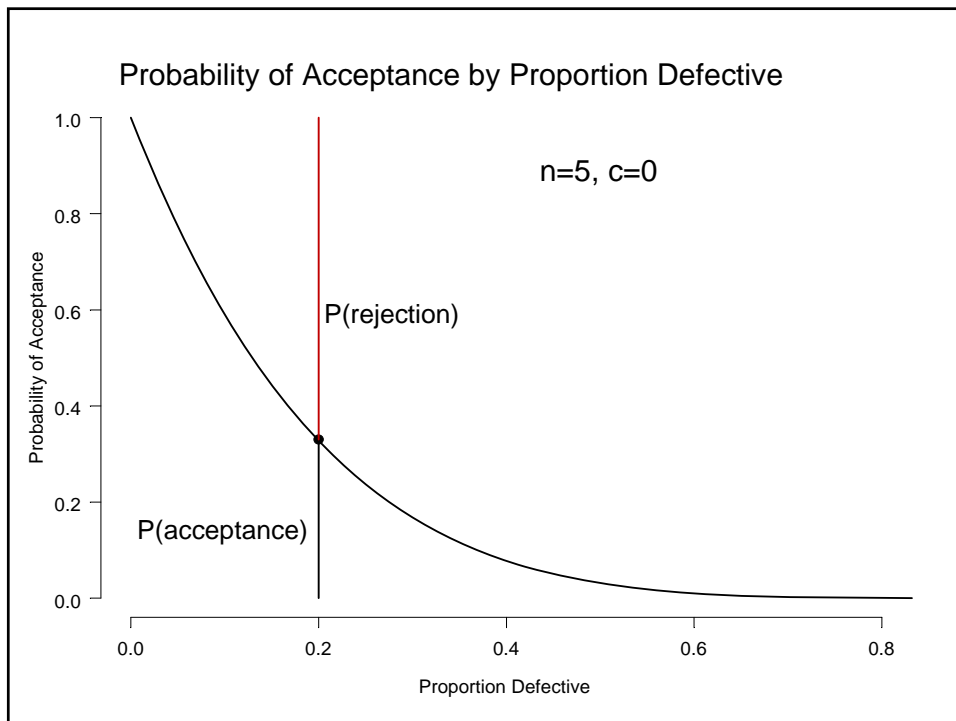
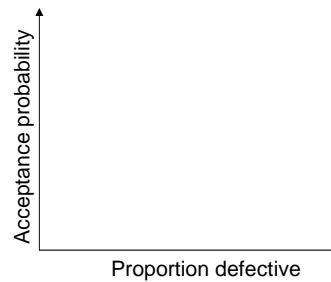
OC Curve for Two-Class Plans

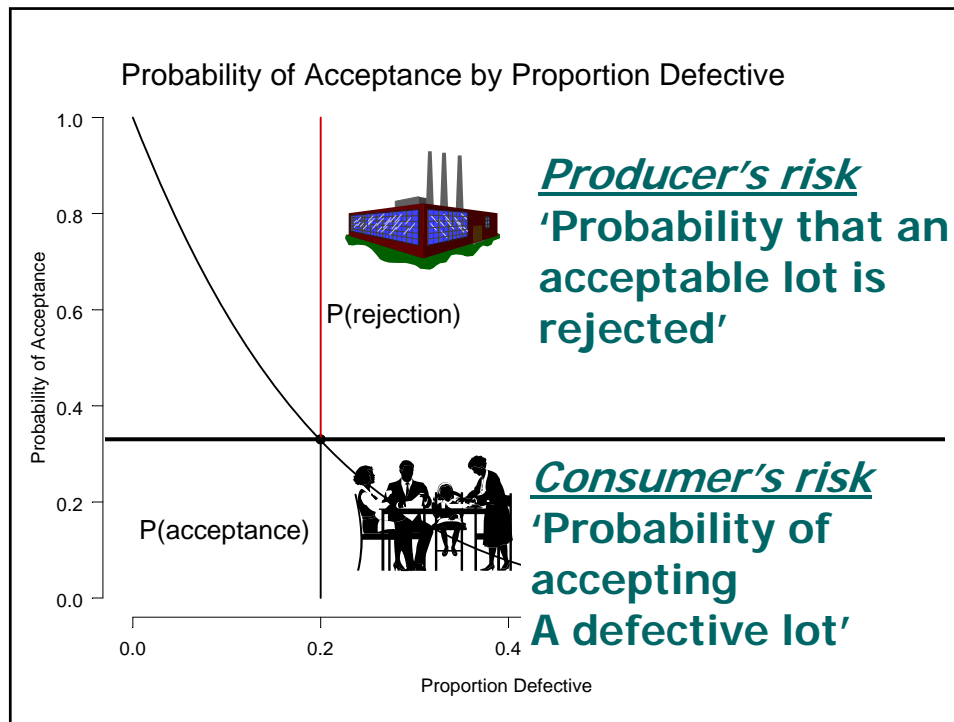
Operation characteristics (OC) or **performance** for two-class sampling plans:

Probability of lot acceptance calculated for possible proportions defective in lot

Plot of OC curve to visualize

- sampling plan performance
- dependency on n and c

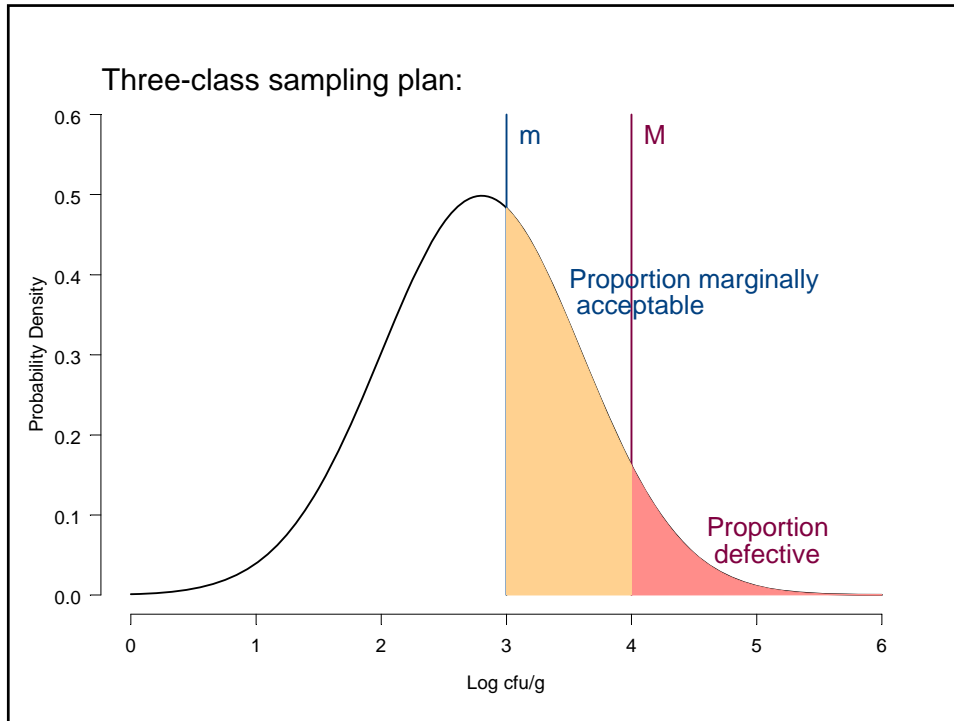




Three-Class Attributes Sampling Plans

Three-class sampling plans consist of

- **n** – number of sample units to be chosen independently and randomly from the lot
- **m** – a microbiological limit that separates good quality from marginally acceptable quality
- **M** – a microbiological limit above which sampling results are unacceptable or defective
- **c** – maximum allowable number of sample units yielding results between **m** and **M** (marginally acceptable); the number of sample units allowed to exceed **M** is usually set to 0



OC Function for Three-Class Plans

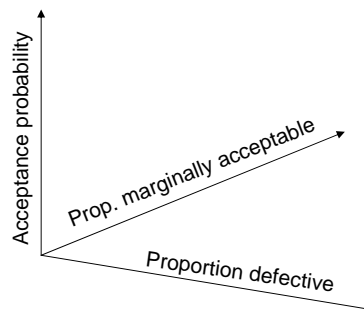
Operation characteristics (OC) or **performance** for three-class plans:

Probability of lot acceptance depending on two proportions

- marginally acceptable: between m and M
- defective: above M

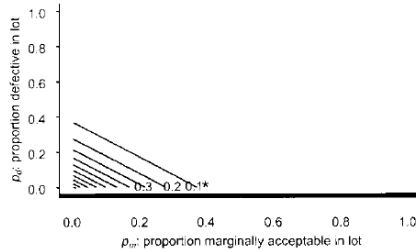


OC function plotted as a three-dimensional graph

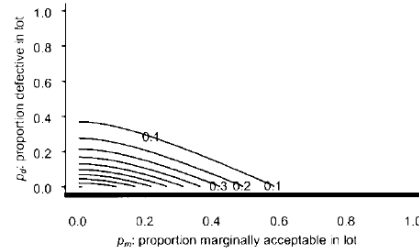


Operating Characteristic Curves, 3-Class Plans

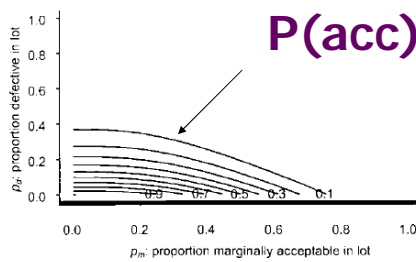
a) 3-class plan: $n=5, c=0$



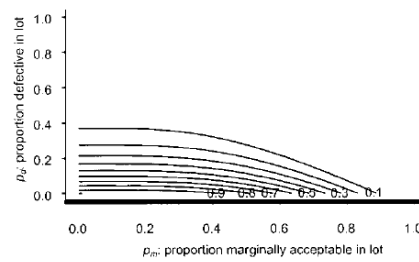
b) 3-class plan: $n=5, c_m=1, c_M=0$



c) 3-class plan: $n=5, c_m=2, c_M=0$



d) 3-class plan: $n=5, c_m=3, c_M=0$

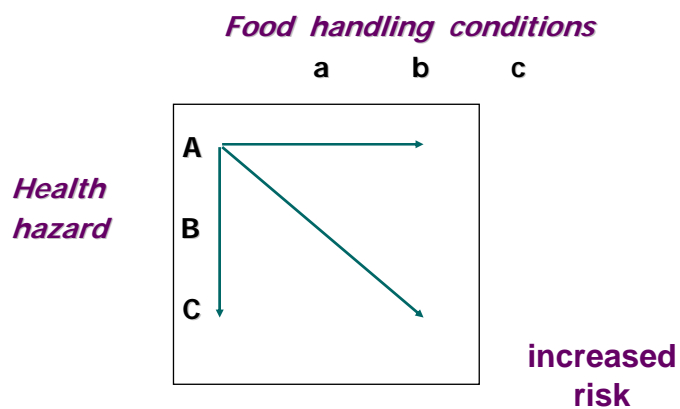


ICMSF Cases

15 cases which reflect:

- Degree of risk
- Conditions of use
- Intended Population

Risk categorization matrix



Categories of hazards

- *A) Moderate:*
 - S. aureus* toxin
 - V. parahaemolyticus*
 - B. cereus*
 - EPEC
- *B) Serious:*
 - Salmonella* (non typhi)
 - Shigella*
 - Listeria monocytogenes*
- *C) Severe:*
 - EHEC (STEC, VTEC)
 - V. cholerae* O1
 - EPEC for infants

Plan Stringency (Case) in Relation to Degree of Health Concern and Conditions of Use.

Type of Hazard	Reduce Degree of Hazard	Cause No Change in Hazard	May Increase Hazard
No direct health hazard			
Utility (general contamination)	Case 1	Case 2	Case 3
Health Hazard			
Low, indirect (indicator)	Case 4	Case 5	Case 6
Moderate, direct, limited spread	Case 7	Case 8	Case 9
Moderate, direct, potentially extensive spread	Case 10	Case 11	Case 12
Severe, direct	Case 13	Case 14	Case 15

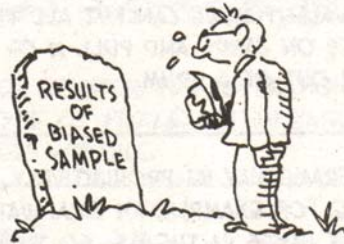
Typical way of expressing performance of sampling plans

Composition of Lot		Number of Sample Units Tested				
% Acceptable	% Defective	5	10	20	60	100
98	2	.90	.82	.67	.30	.13
95	5	.77	.60	.36	.05	.01
90	10	.59	.35	.12	<	<
80	20	.17	.11	.01		
70	30	.03	.03	<		
50	50	.01	<			
40	60	<				
30	70					

Two-Class Plans (c=0): Probabilities of Acceptance

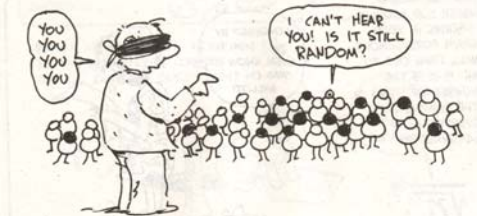
Important Properties of Sampling

THE SIMPLE RANDOM SAMPLE HAS TWO PROPERTIES THAT MAKE IT THE STANDARD AGAINST WHICH WE MEASURE ALL OTHER METHODS:



1) UNBIASED: EACH UNIT HAS THE SAME CHANCE OF BEING CHOSEN.

2) INDEPENDENCE: SELECTION OF ONE UNIT HAS NO INFLUENCE ON THE SELECTION OF OTHER UNITS.



Gonick & Smith, Harper Resource, 1993.

Microbial Sampling is a Bernoulli Trial

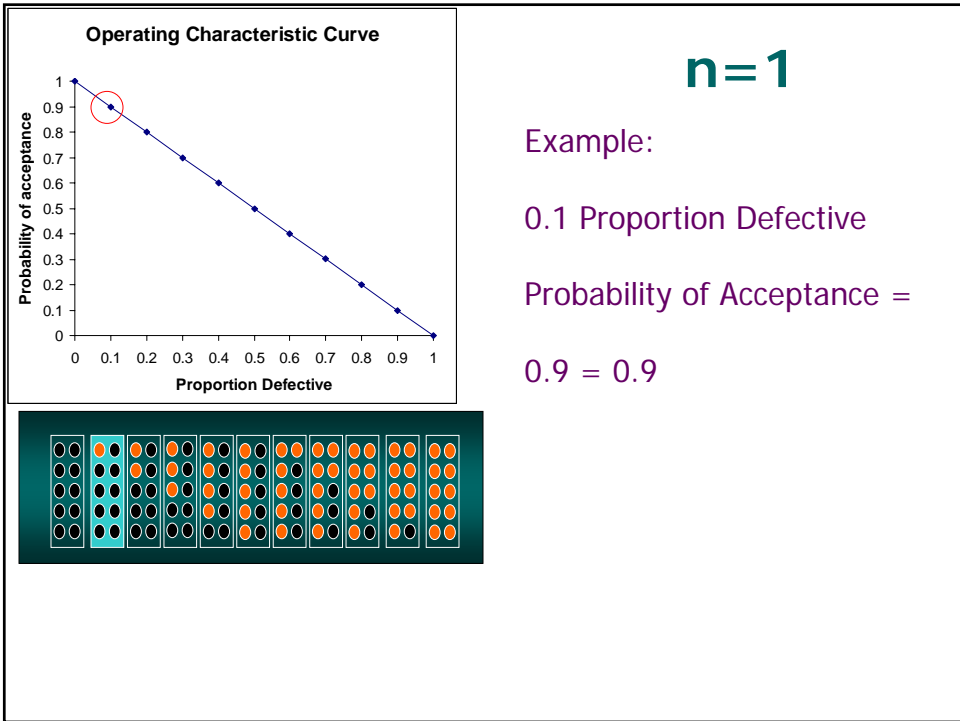
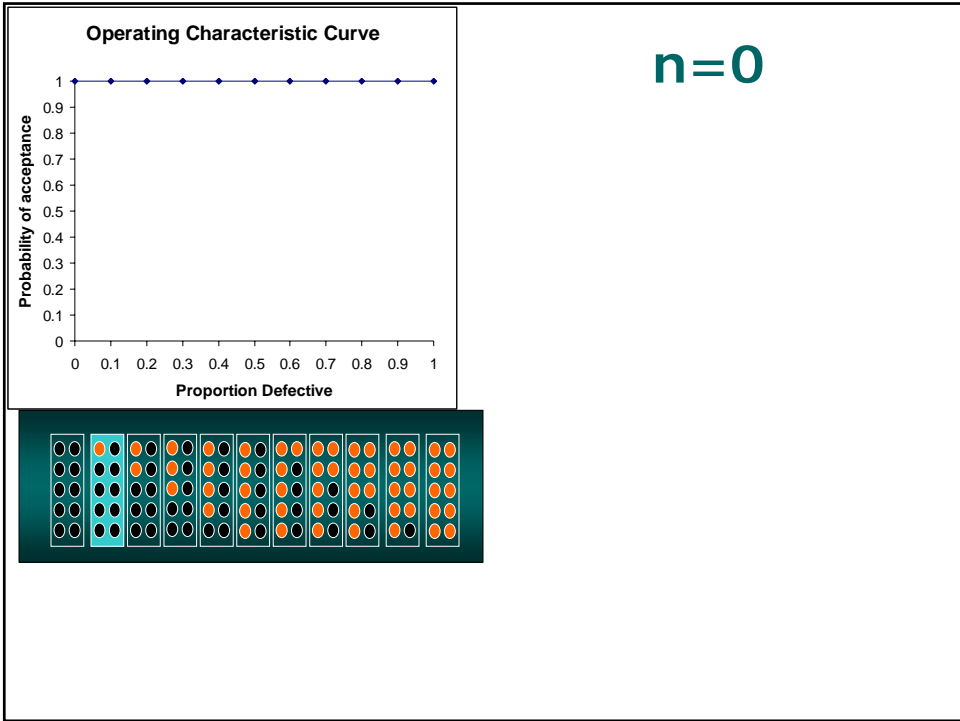
Bernoulli trial,

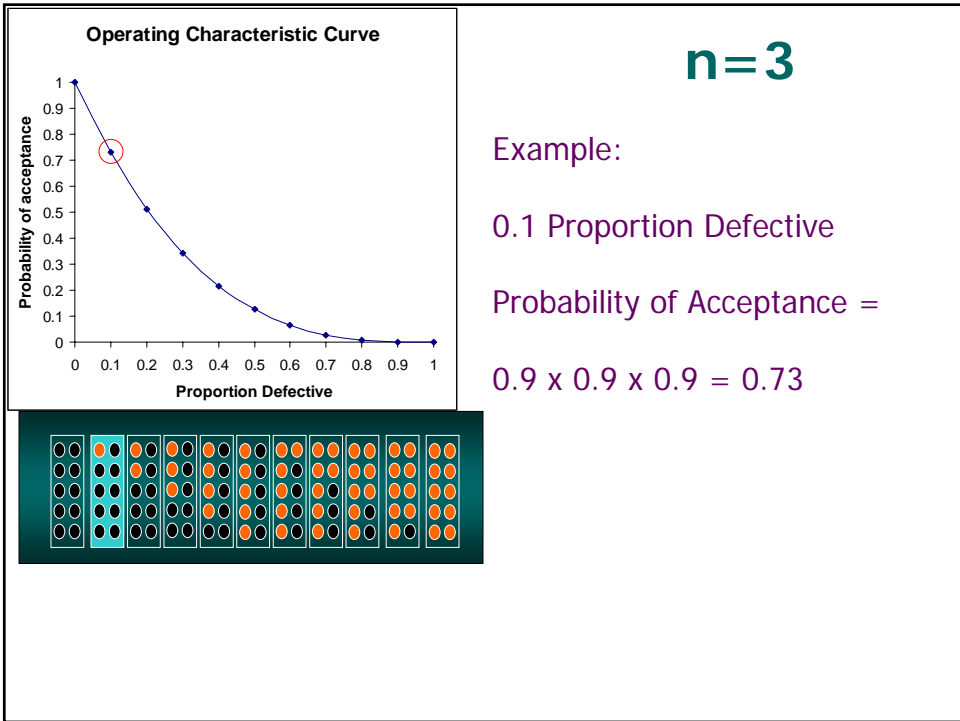
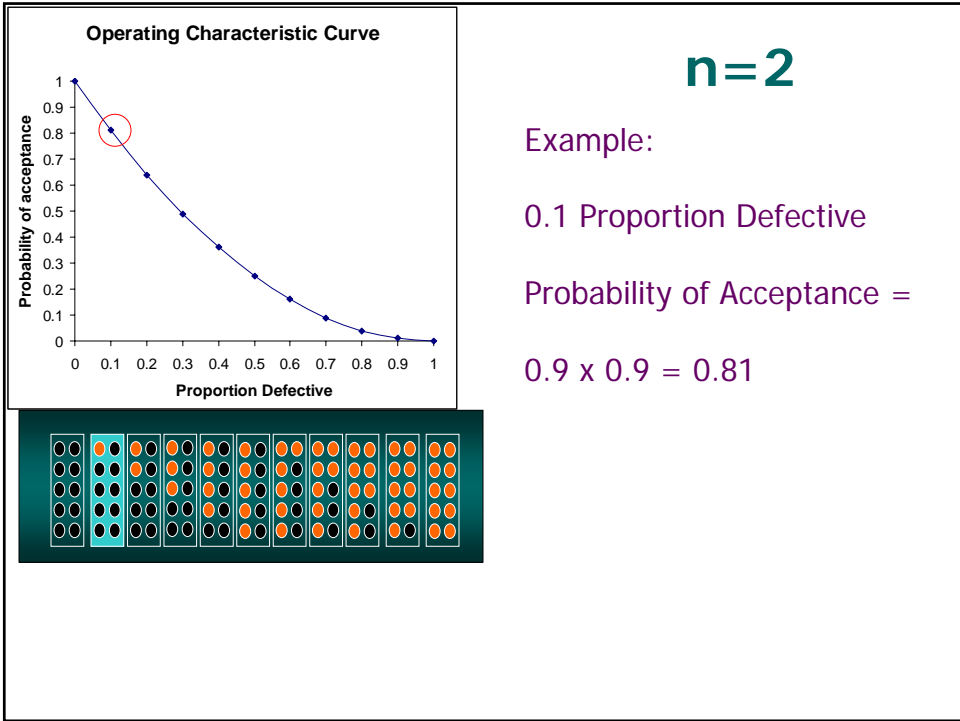
PROVIDED IT HAS THESE CRITICAL PROPERTIES:

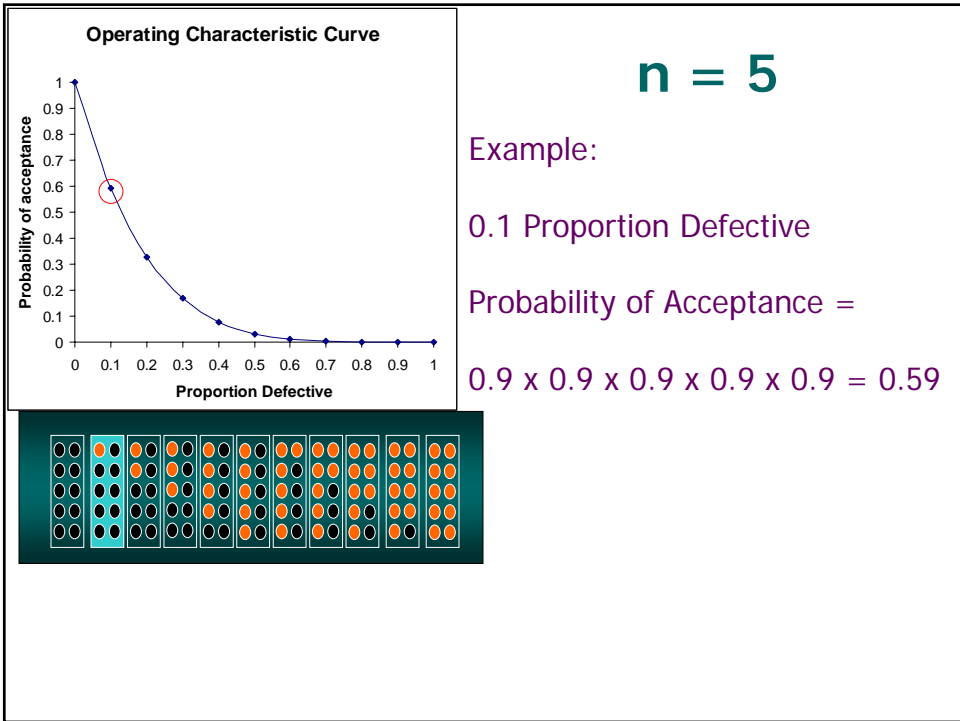
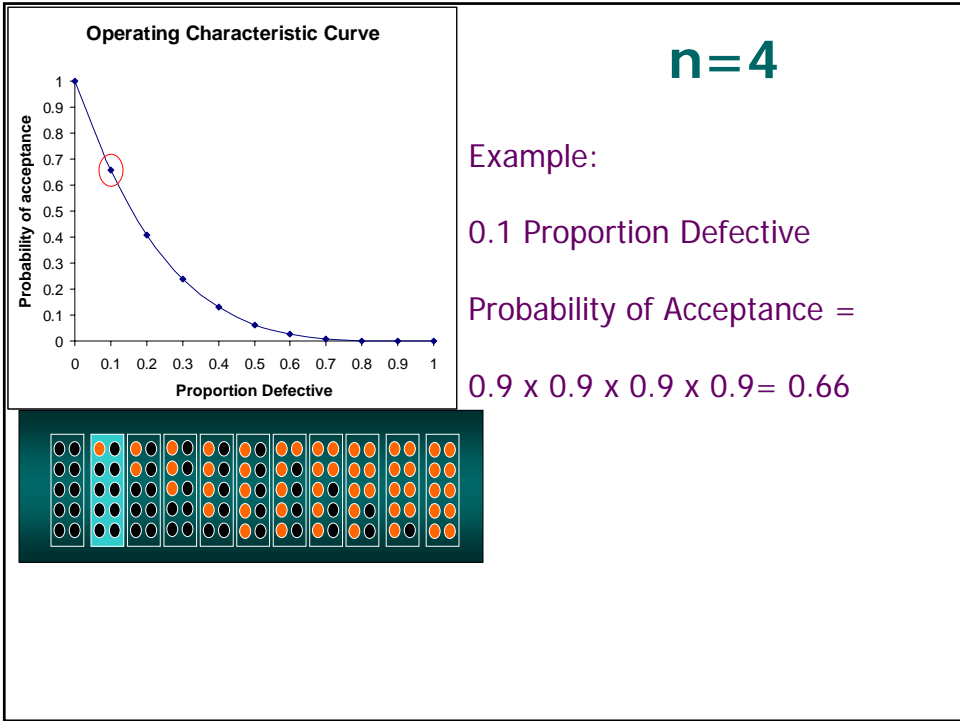
- 1) THE RESULT OF EACH TRIAL MAY BE EITHER A SUCCESS OR A FAILURE
- 2) THE PROBABILITY p OF SUCCESS IS THE SAME IN EVERY TRIAL.
- 3) THE TRIALS ARE INDEPENDENT: THE OUTCOME OF ONE TRIAL HAS NO INFLUENCE ON LATER OUTCOMES.

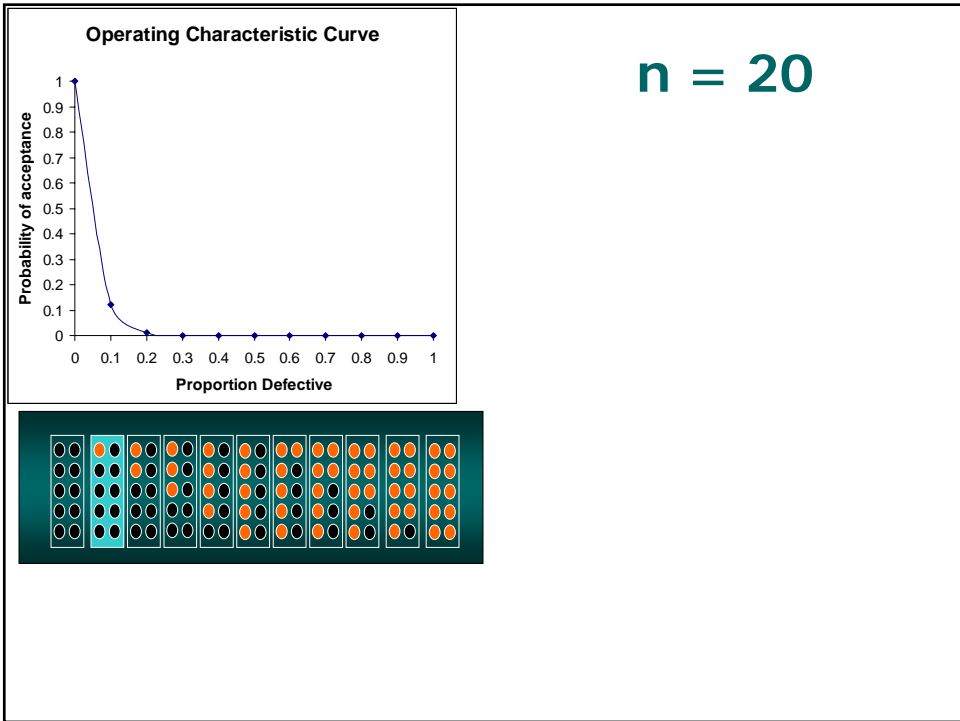
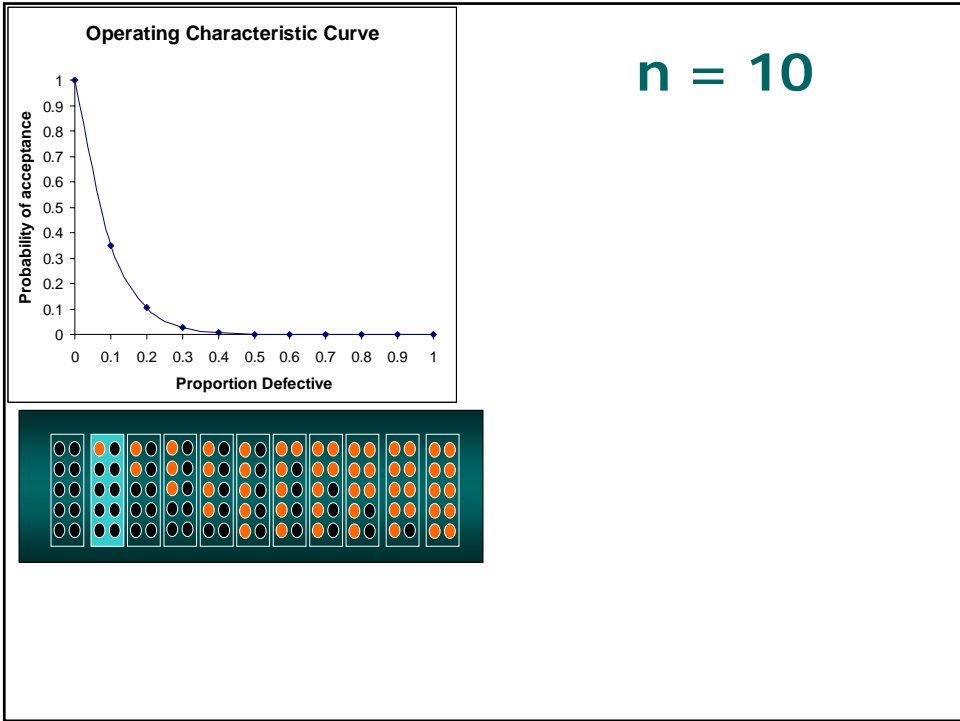


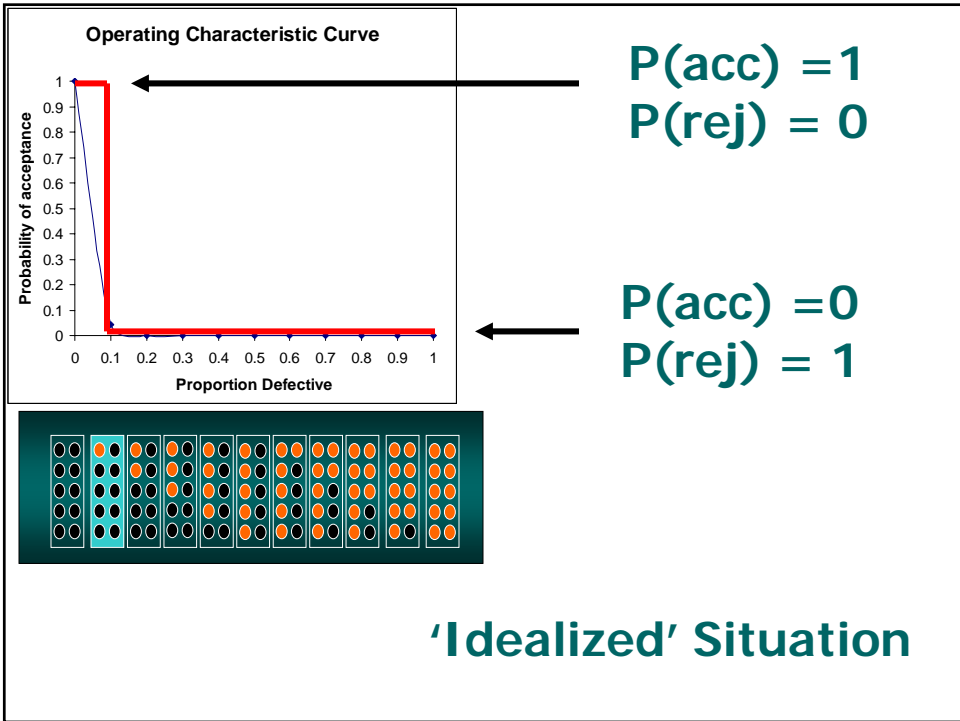
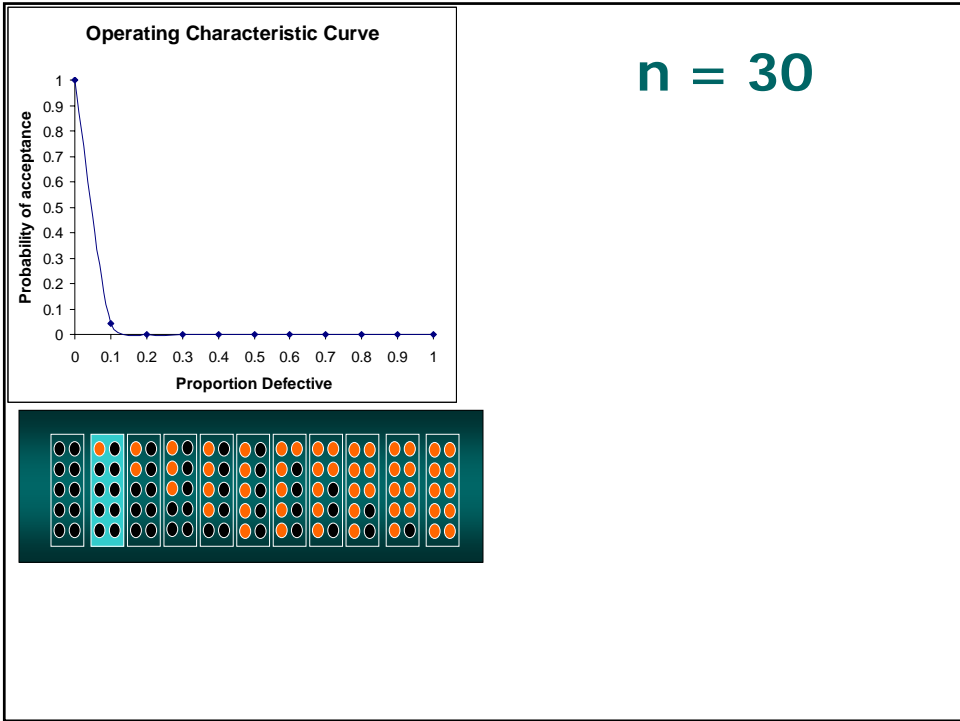
Gonick & Smith, Harper Resource, 1993.

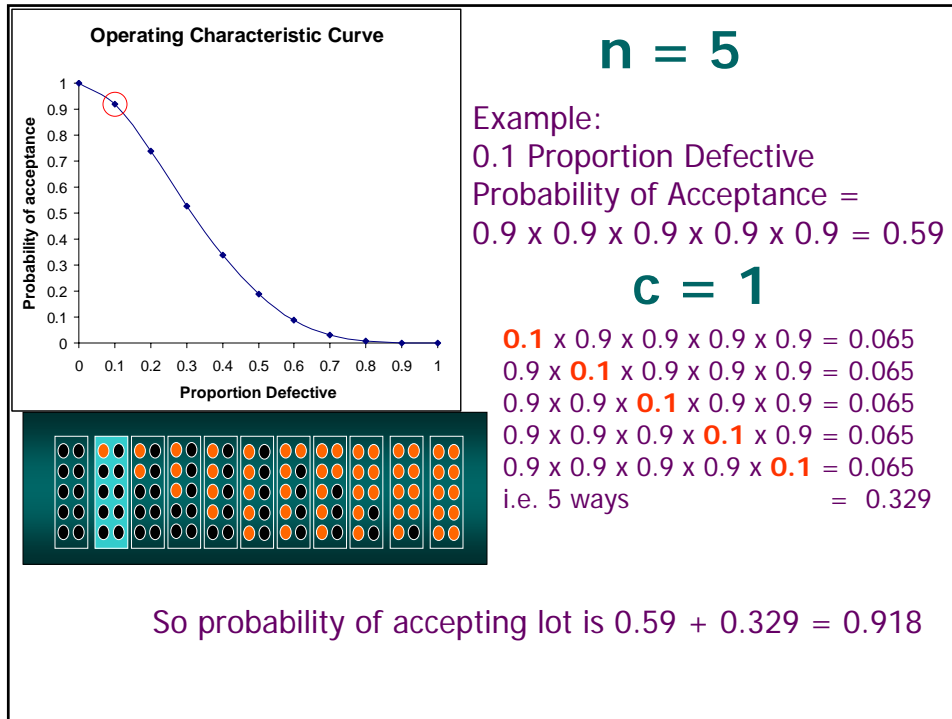












Performance of sampling plans and concentration controlled

Alternative approach for quantitative data:

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

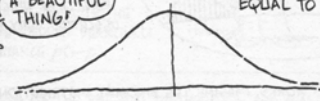
"Fuzzy Central Limit Theorem":

DATA THAT ARE INFLUENCED BY MANY SMALL AND UNRELATED RANDOM EFFECTS ARE APPROXIMATELY NORMALLY DISTRIBUTED.

$$f(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}$$

THIS FUNCTION IS CALLED THE **standard normal distribution**.

(e IS A USEFUL MATHEMATICAL CONSTANT APPROXIMATELY EQUAL TO 2.718.)



Gonick & Smith, Harper Resource, 1993.

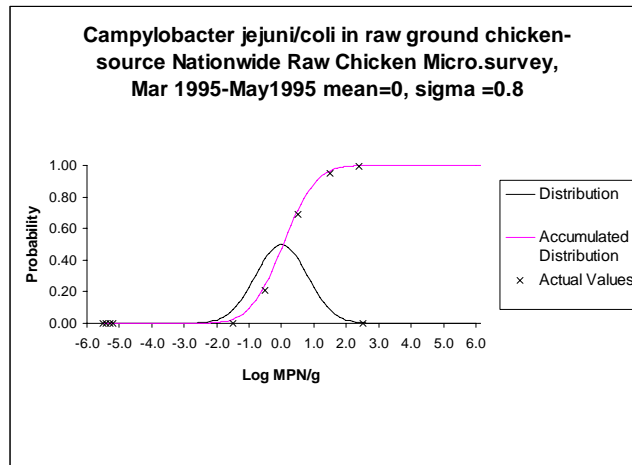
'When the error is proportional to the measurement the use of logarithms is likely to produce normal curves.....'

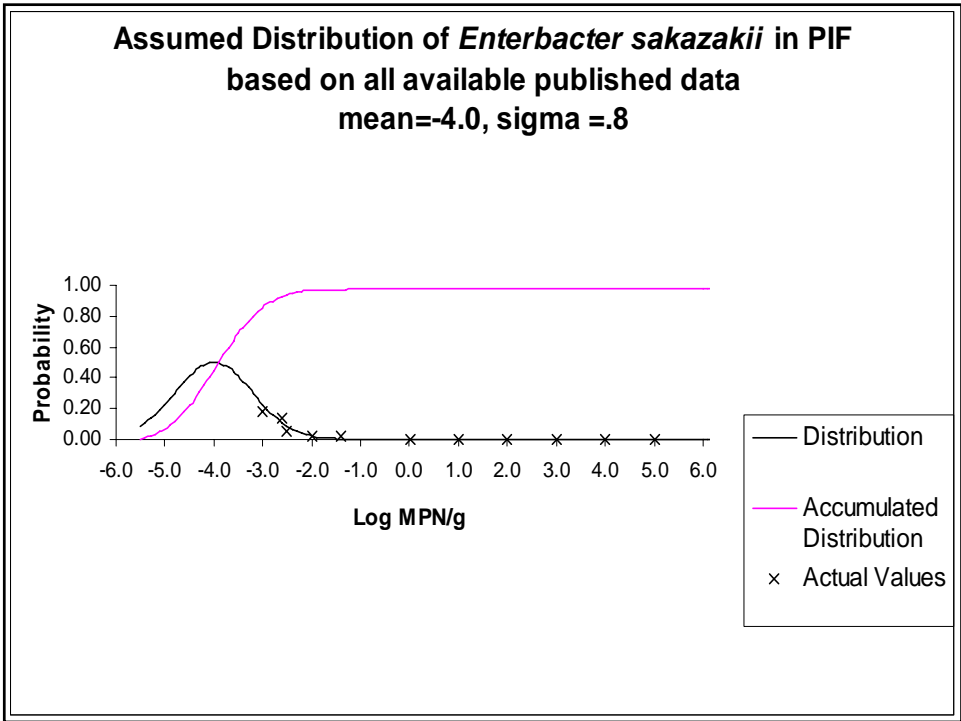
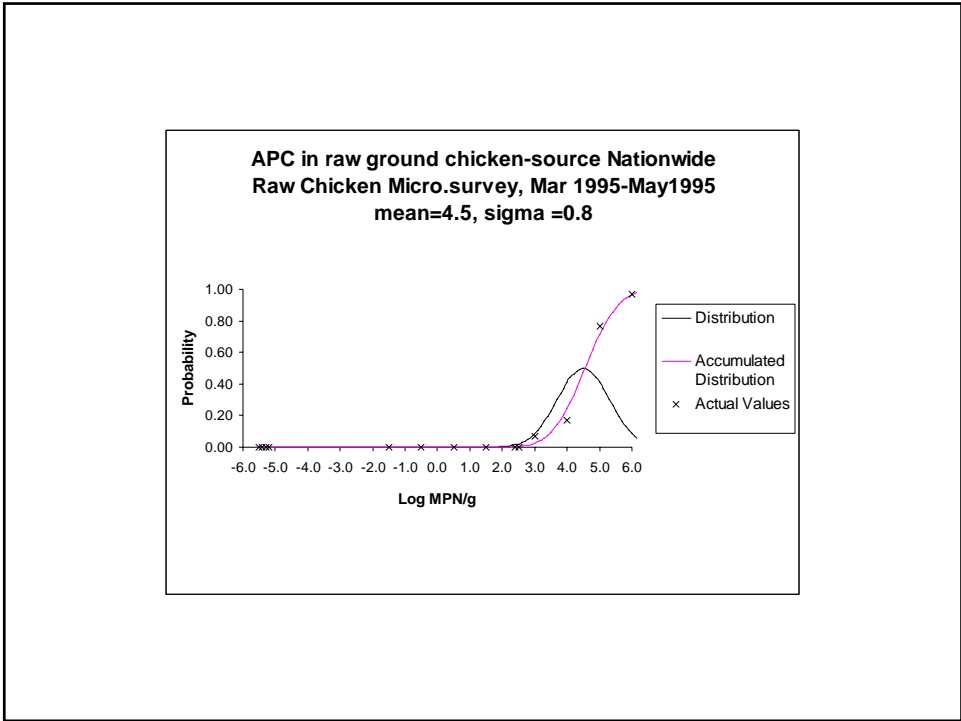
Gaddum, Nature No.3964, October 20, 1945

Log Normality of Total Viable Counts in Batches of Foods

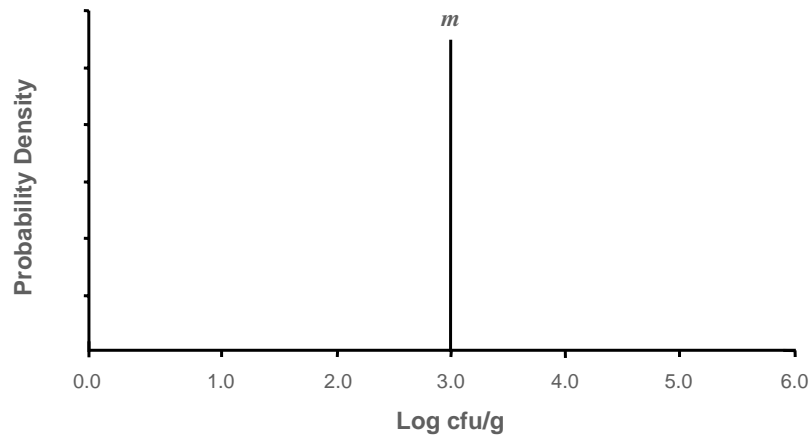
Commodity	No. of Suppliers	No. of batches NOT LOG NORMAL	No. of batches Examined	% of batches NOT LOG NORMAL
Frozen fish	2	35	518	6.7
Frozen crust.	1	31	393	7.9
Frozen meat	2	13	159	8.2
Frozen veg	1	1	41	2.4
Frozen dairy	1	5	52	9.6
Powdered	2	15	118	12.7
Overall	6	100	1281	7.8

Fillibens (1975), Techometrics, 17, 111-117

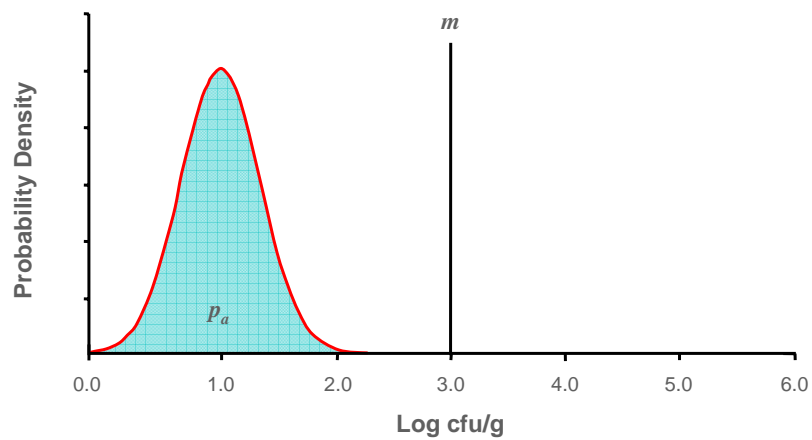


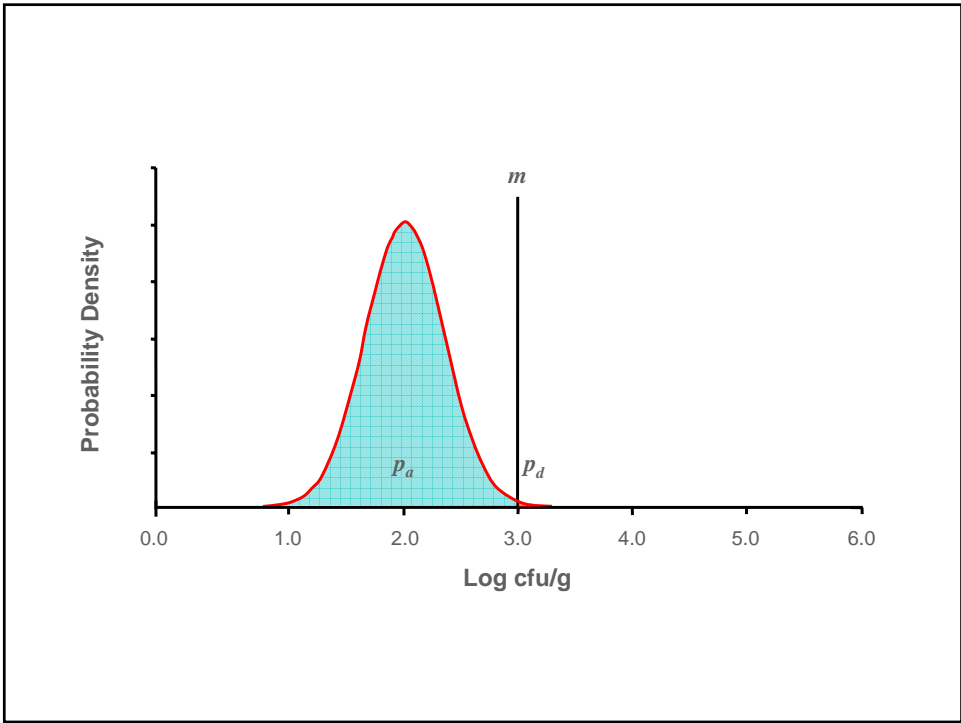
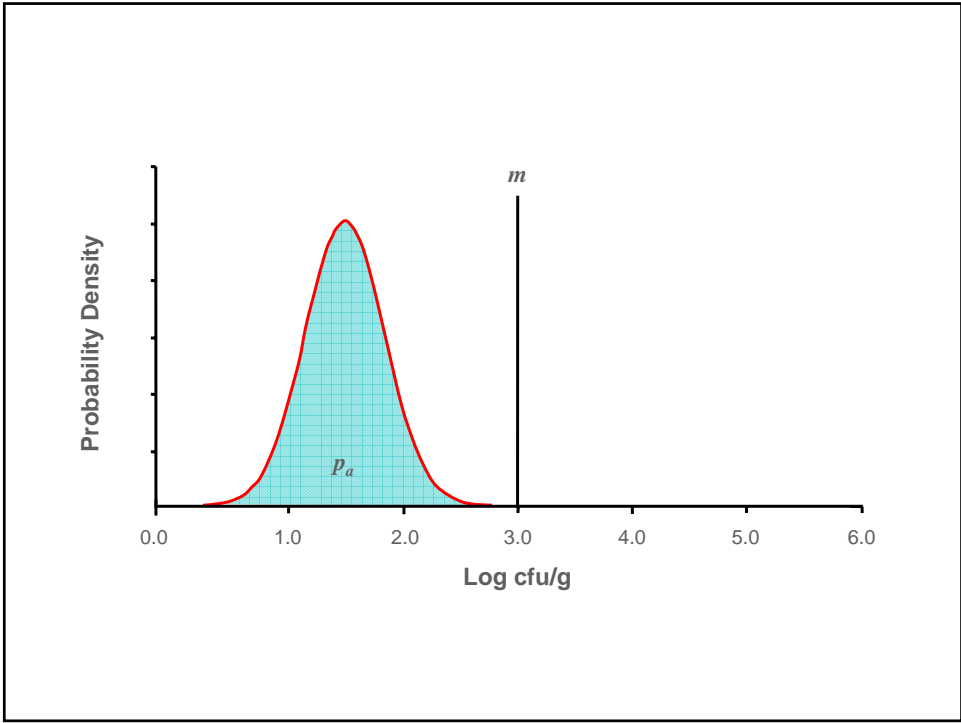


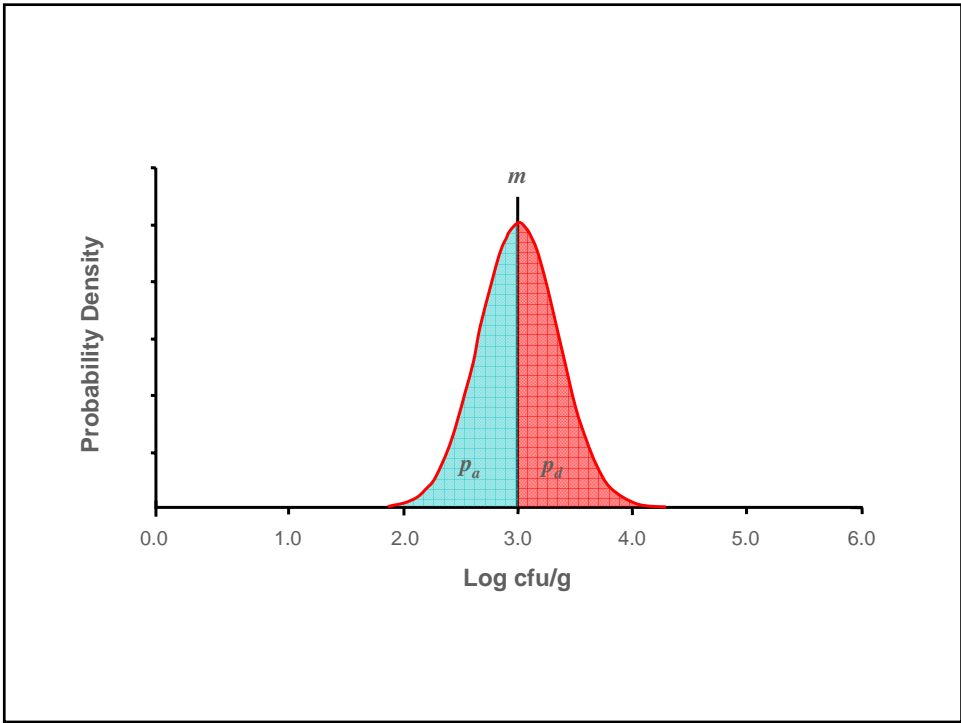
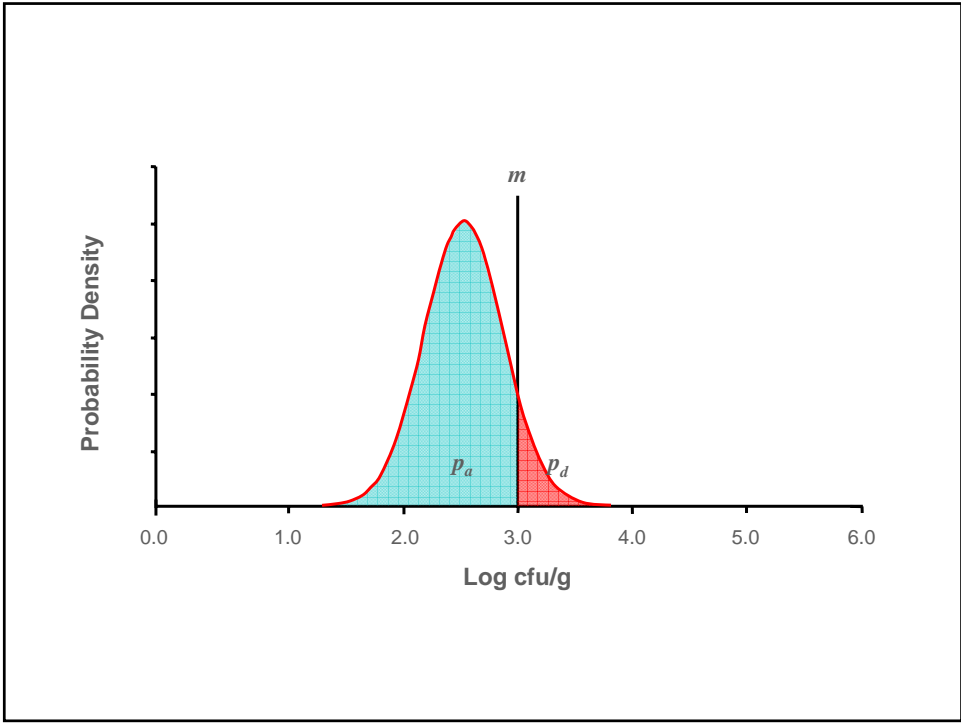
Linking the Performance of attribute sampling plans to the concentration of bacteria controlled

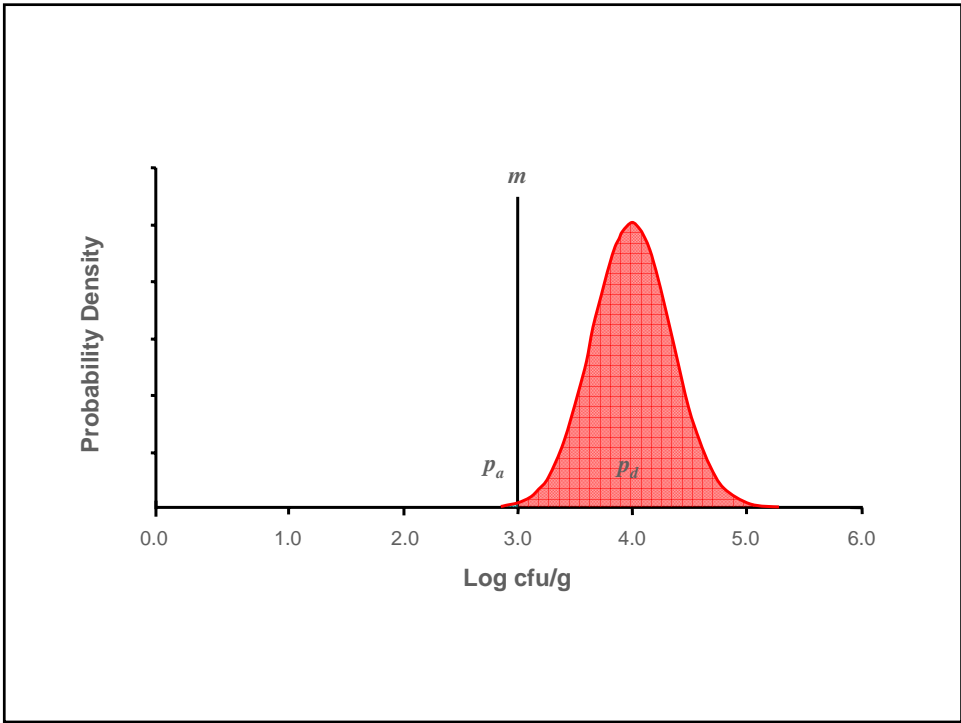
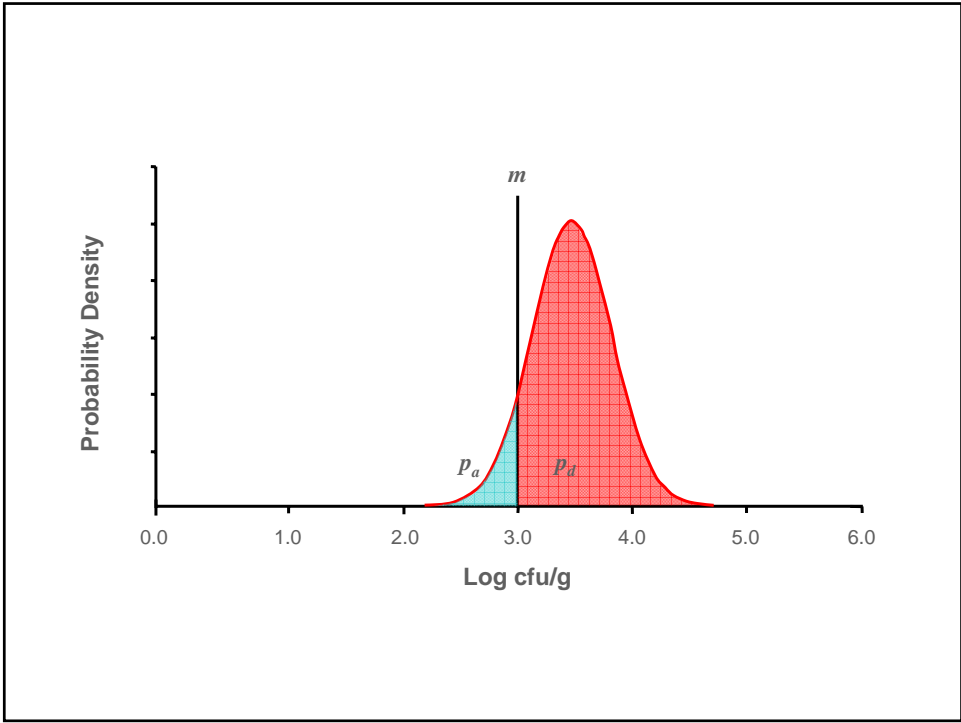


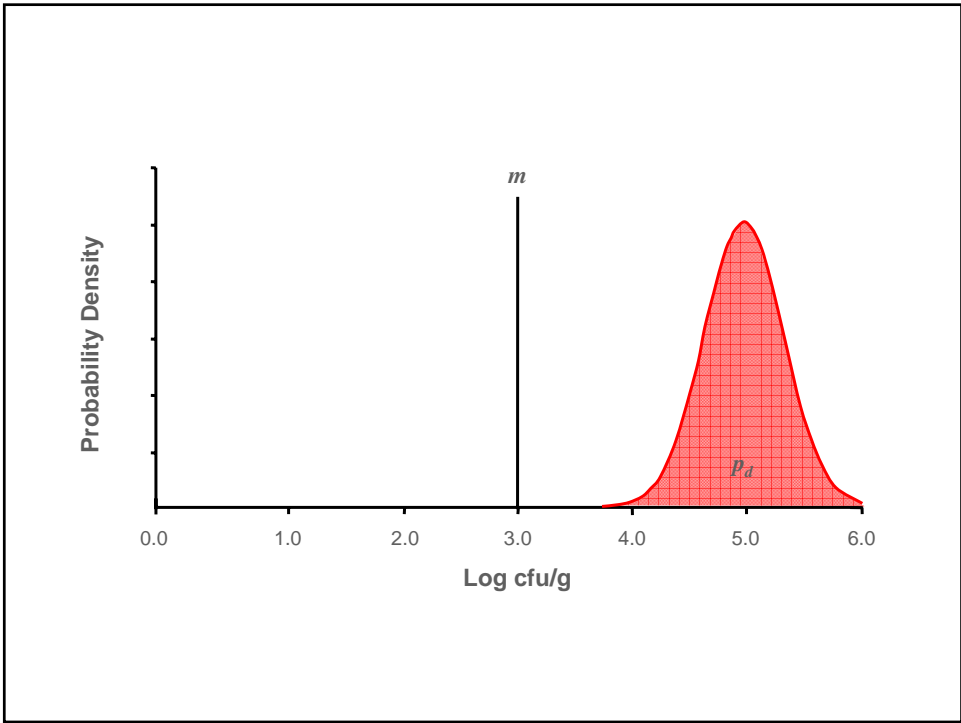
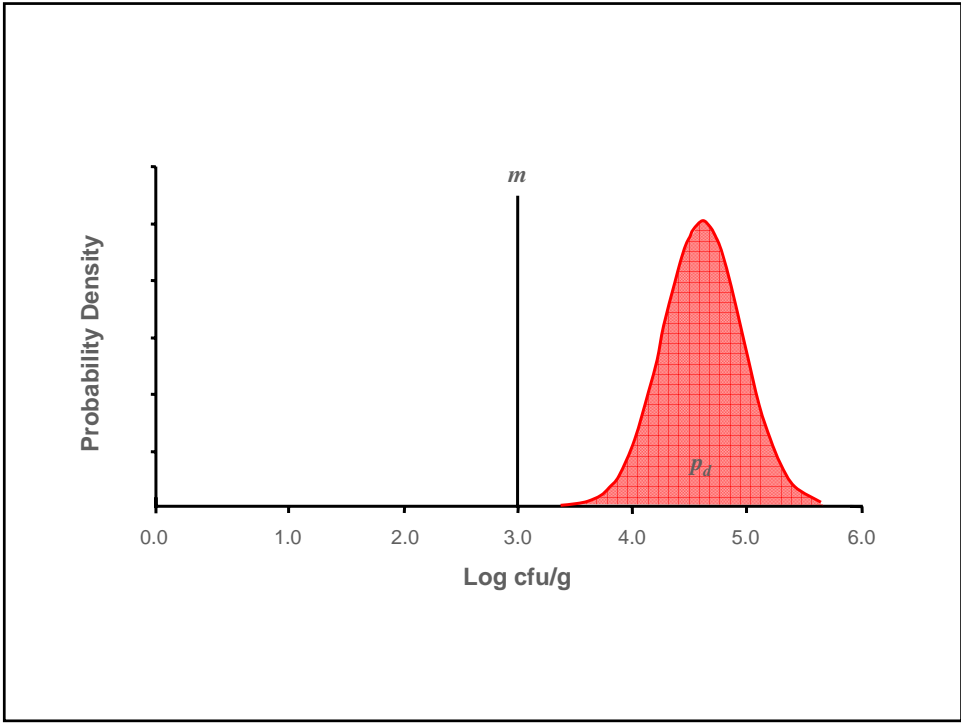
Proportion Defective

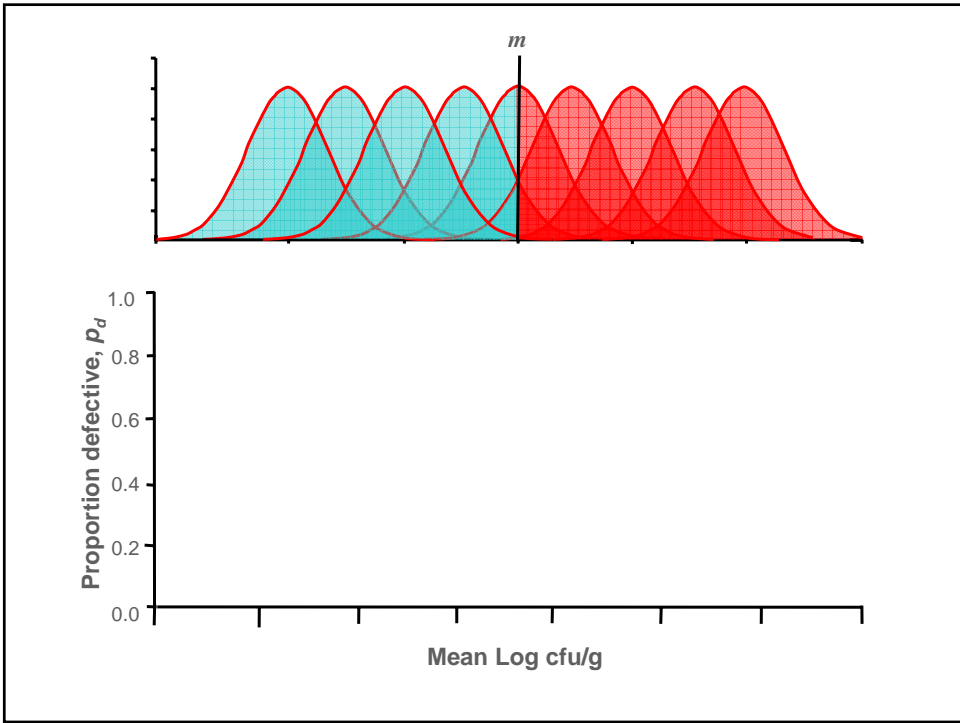
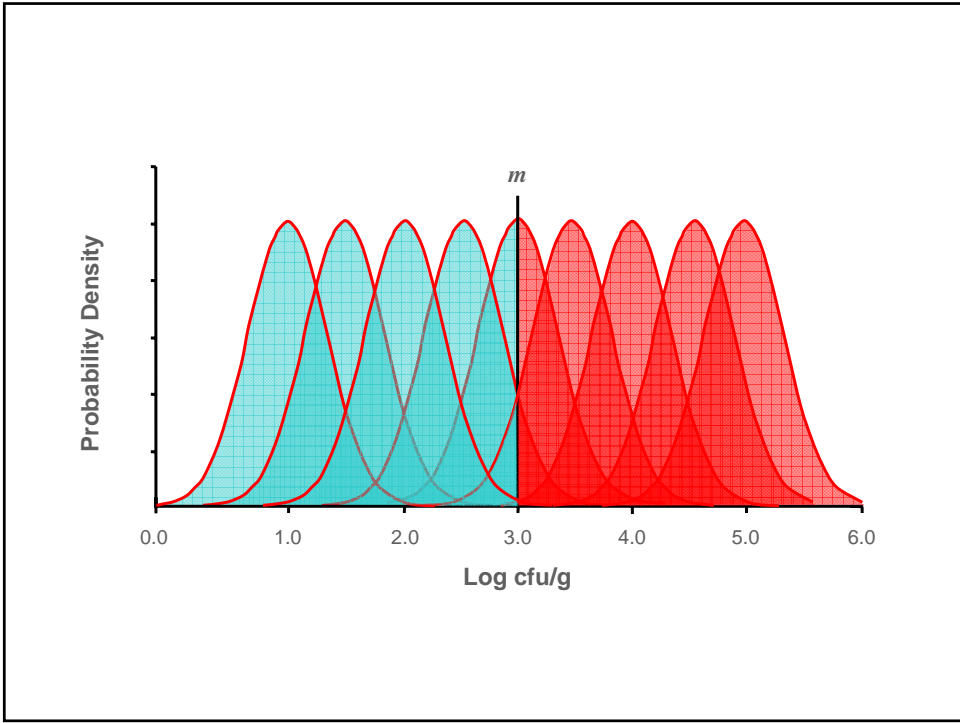


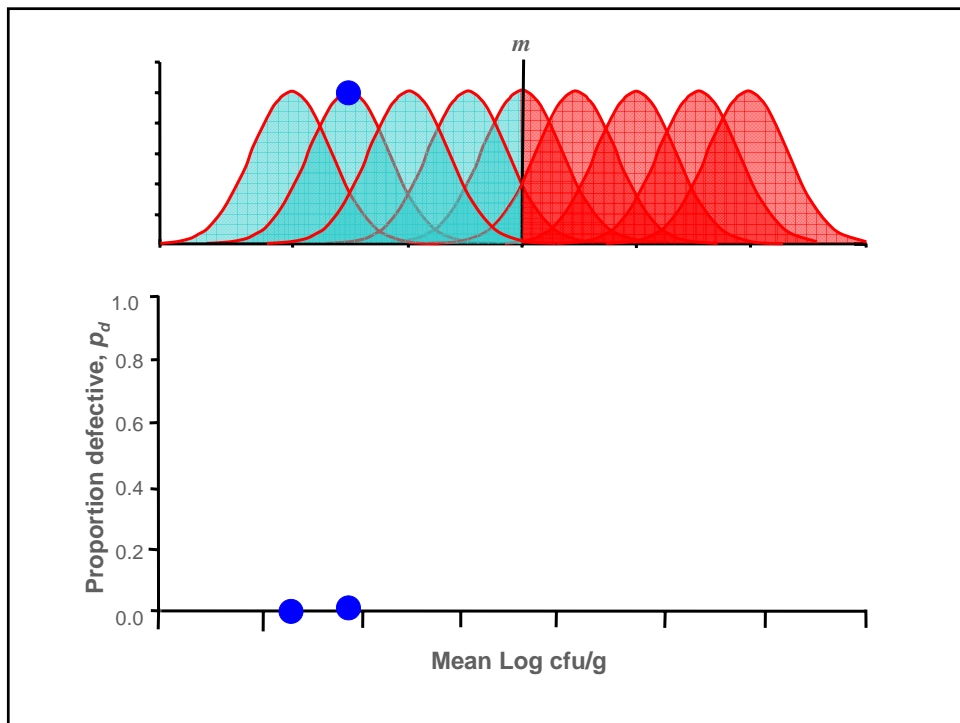
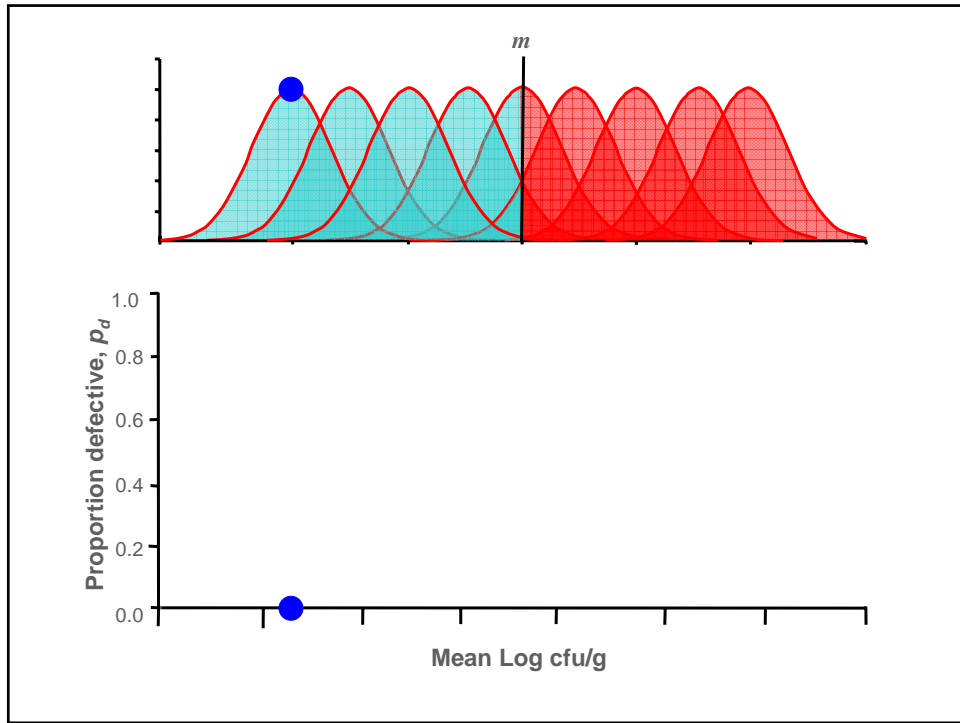


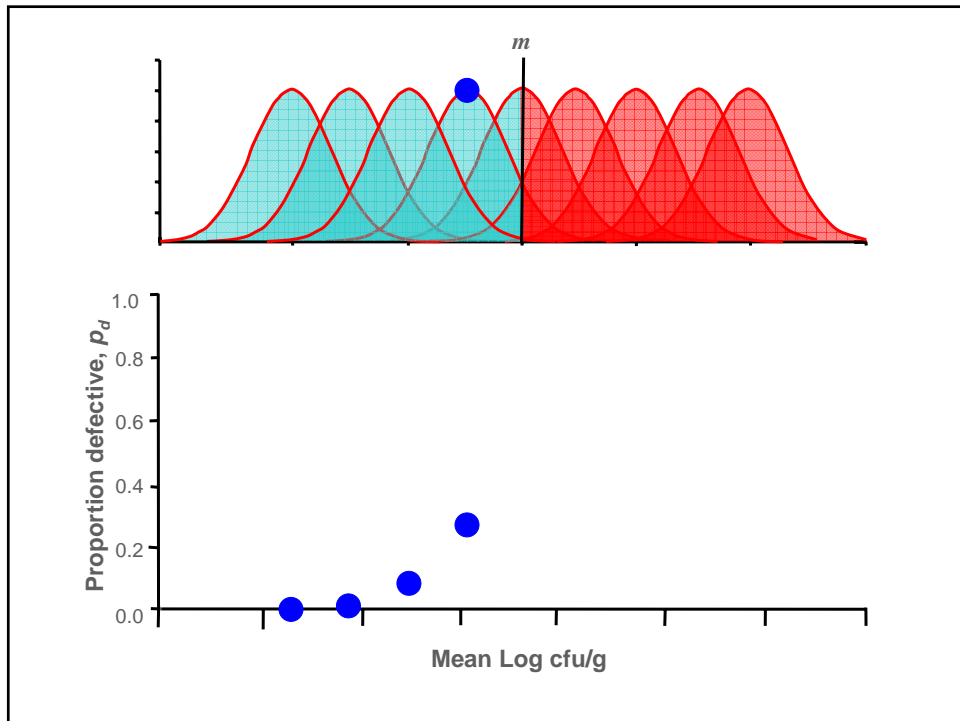
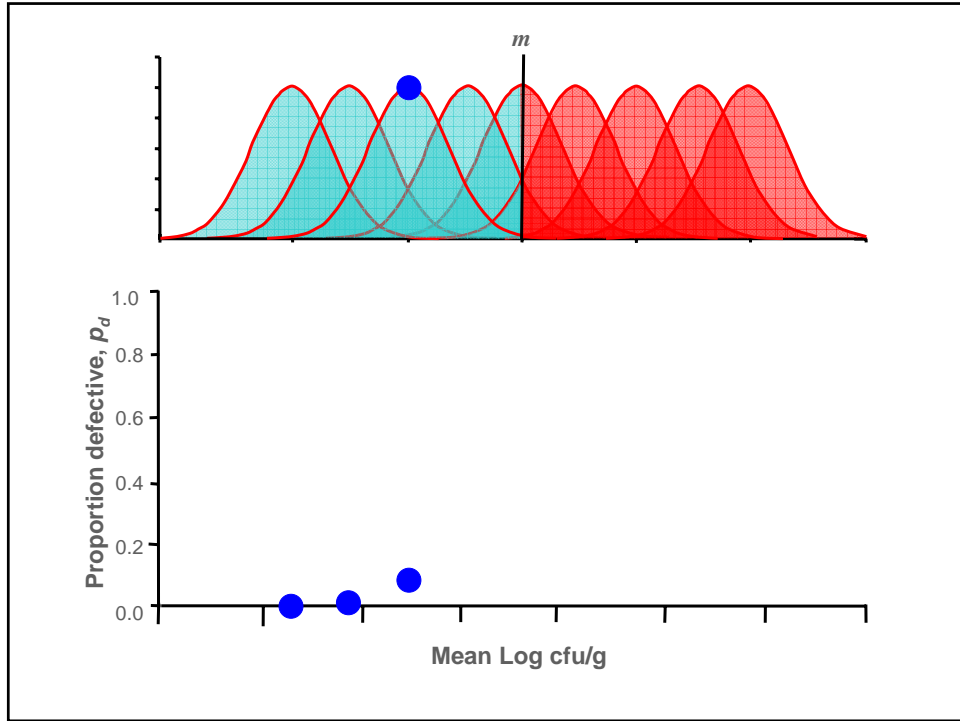


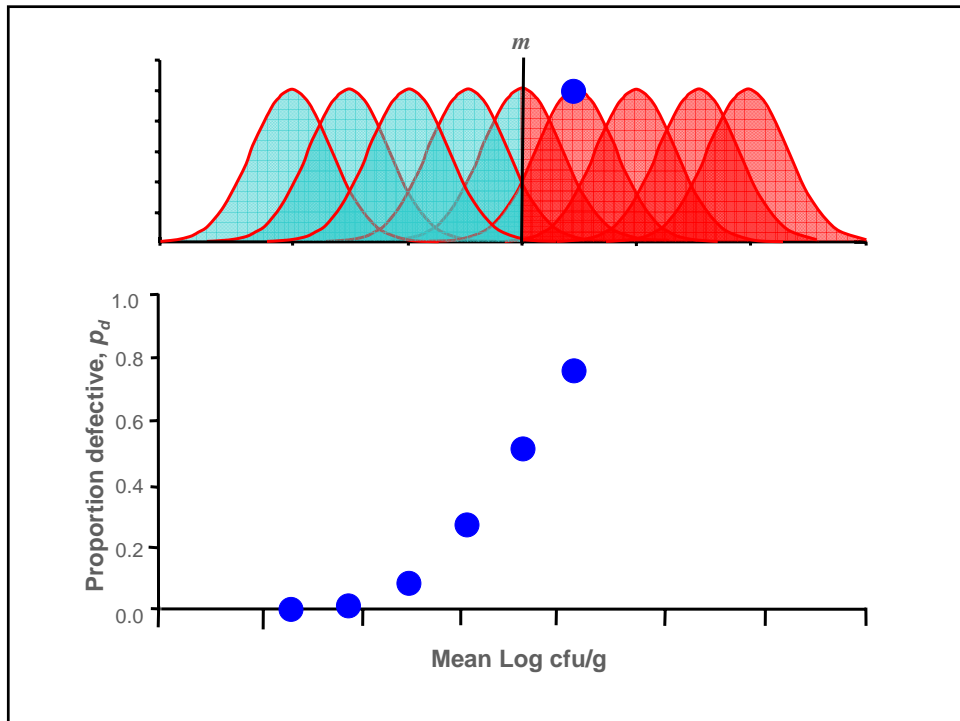
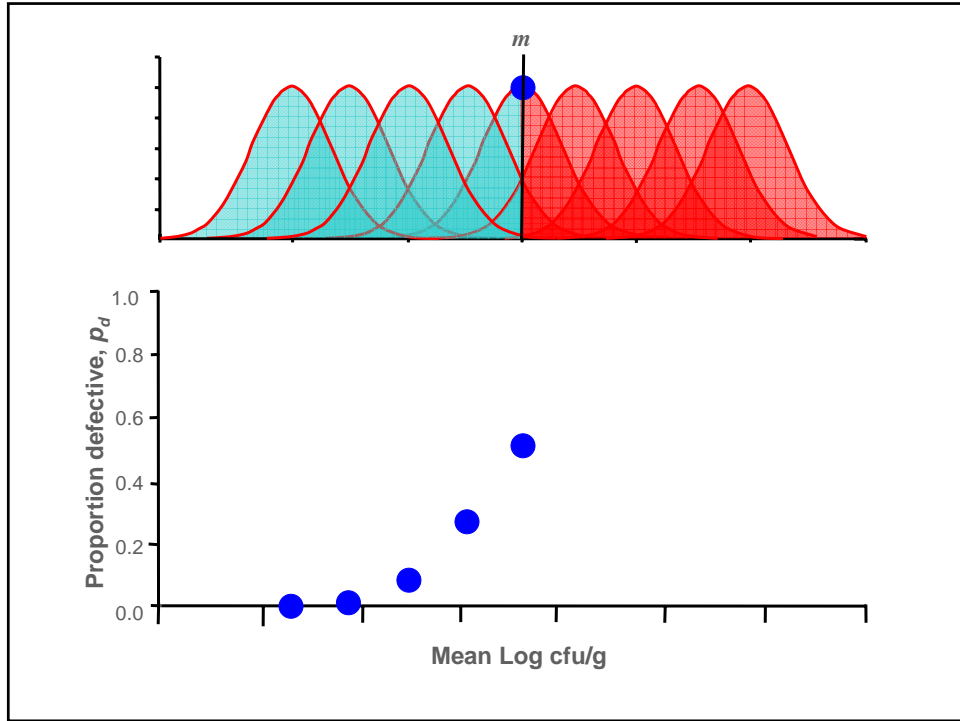


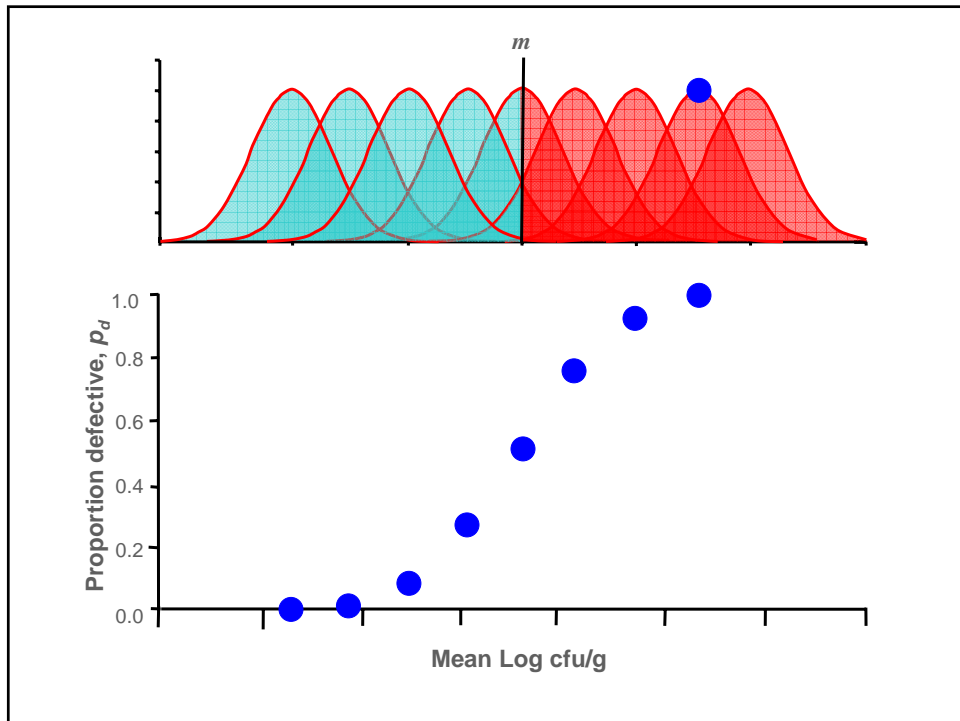
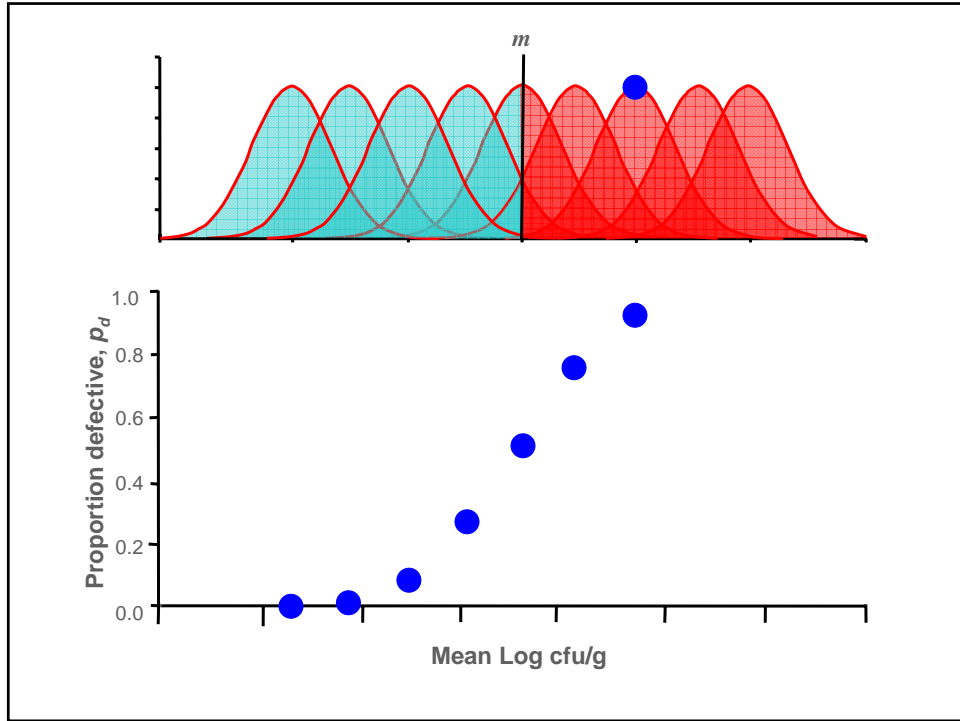


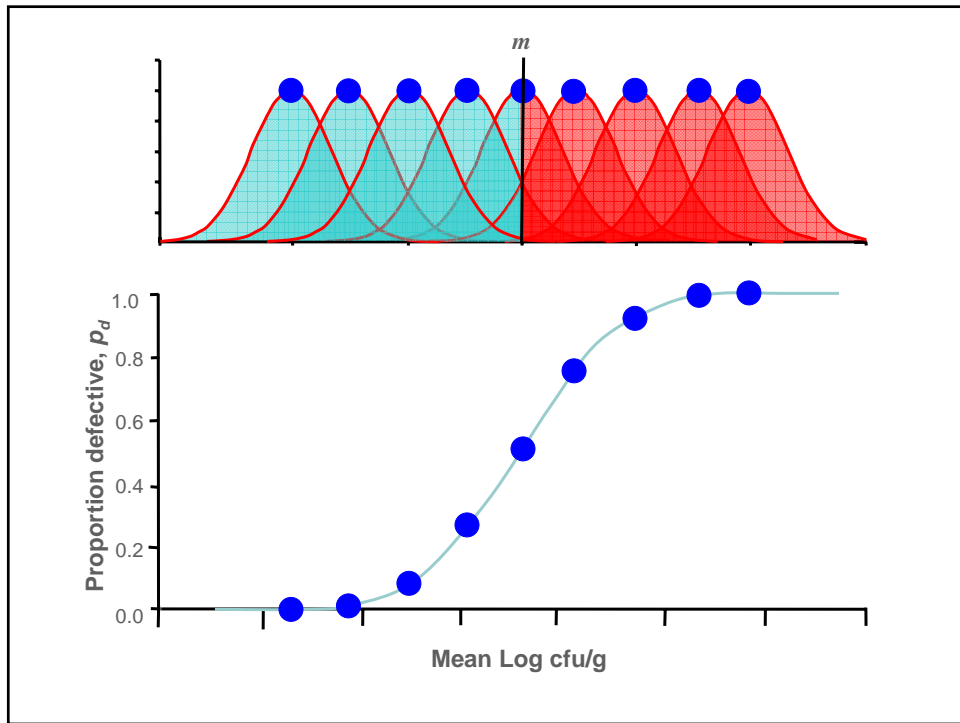
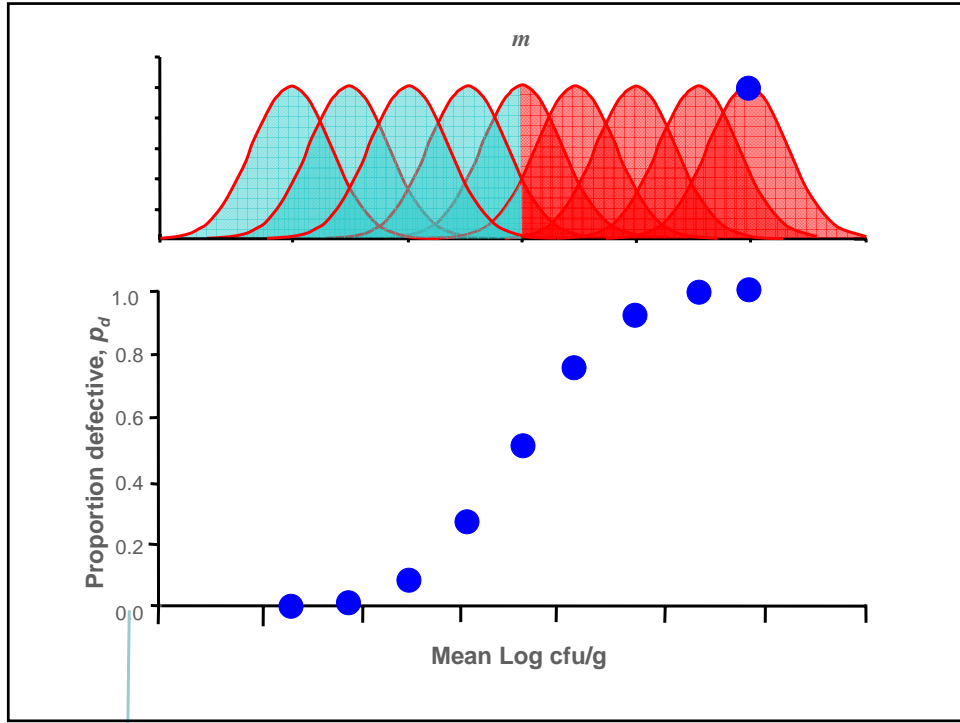


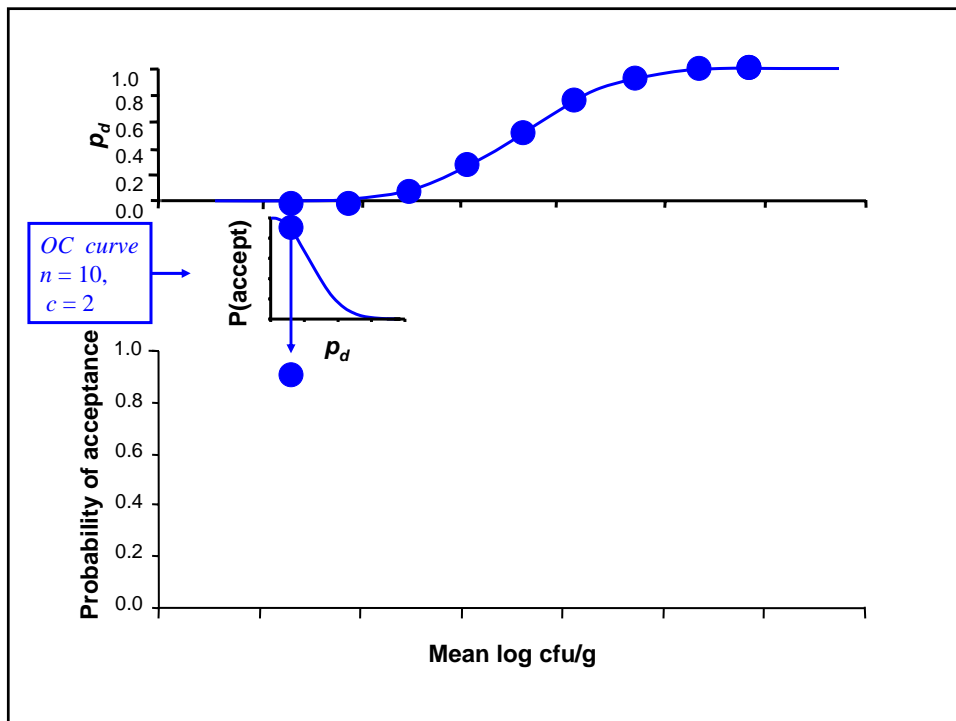
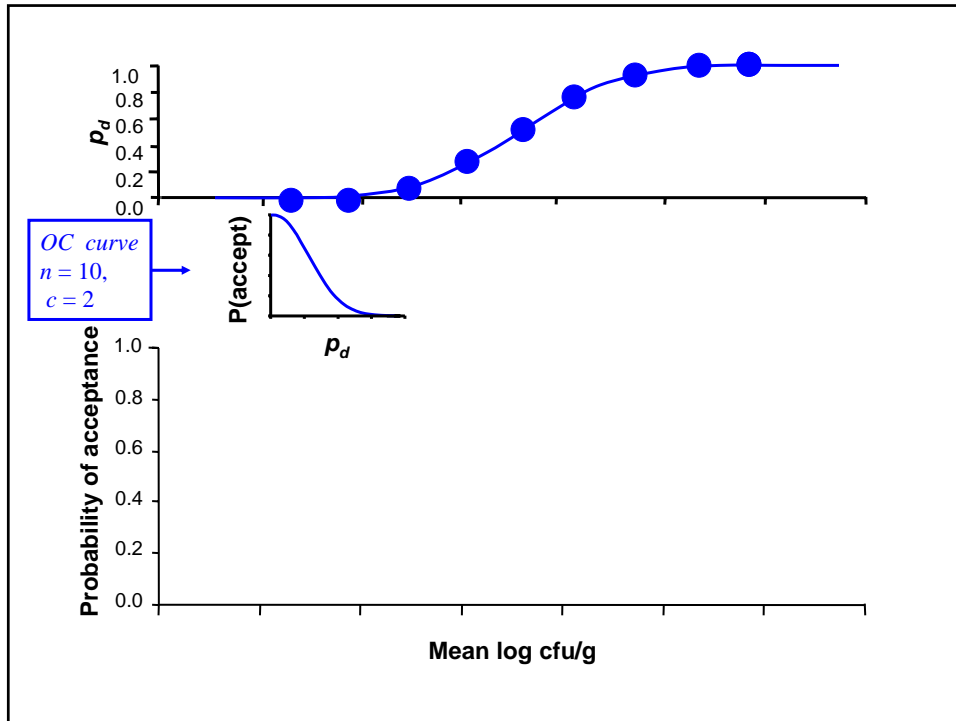


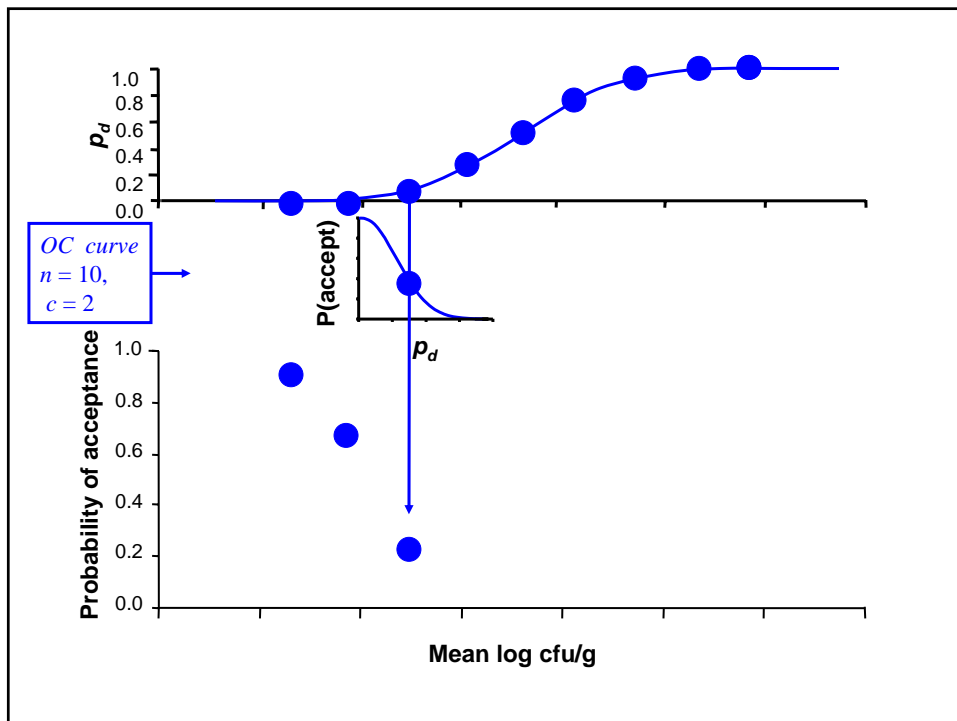
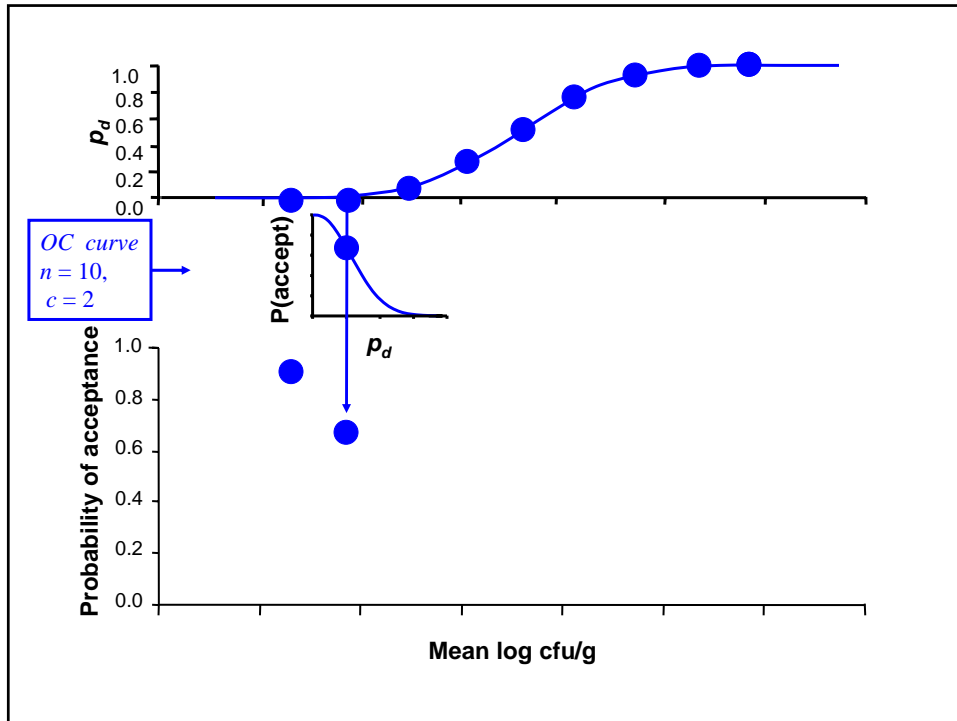


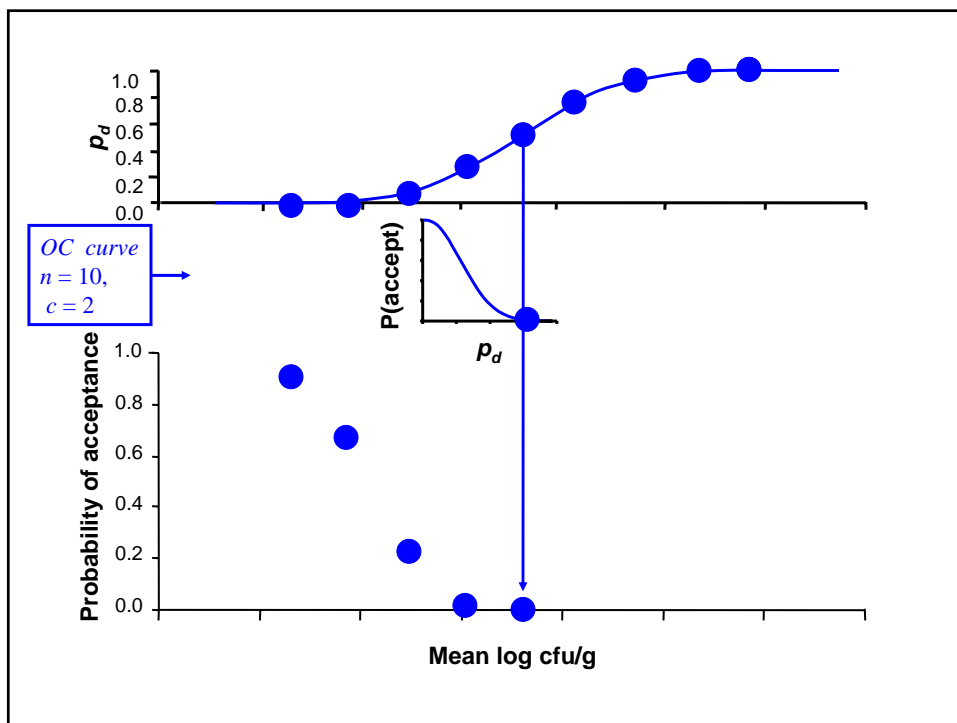
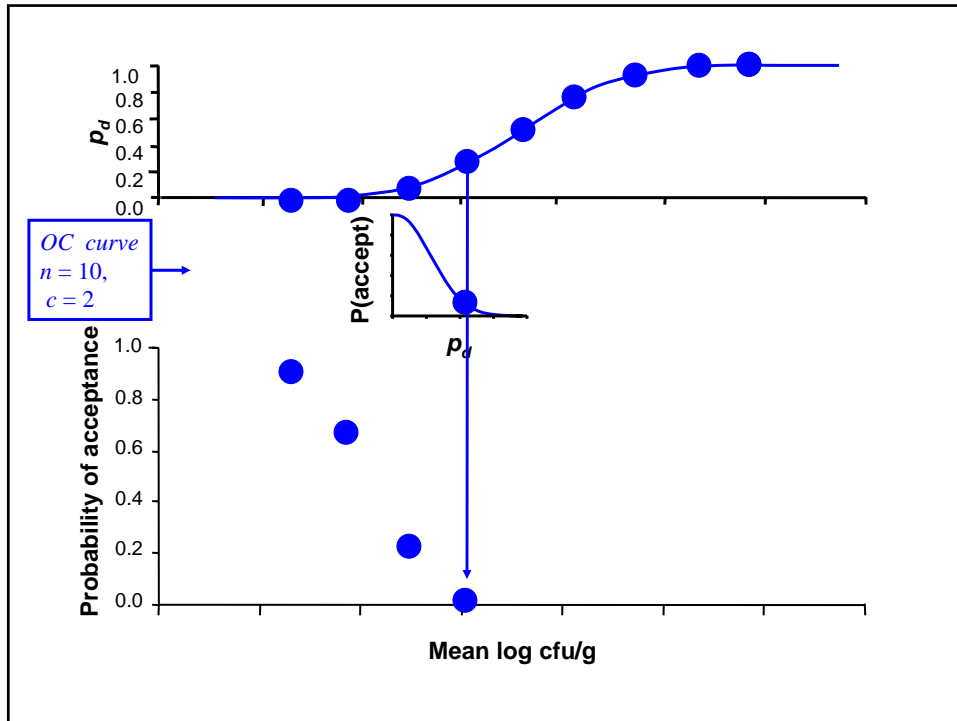


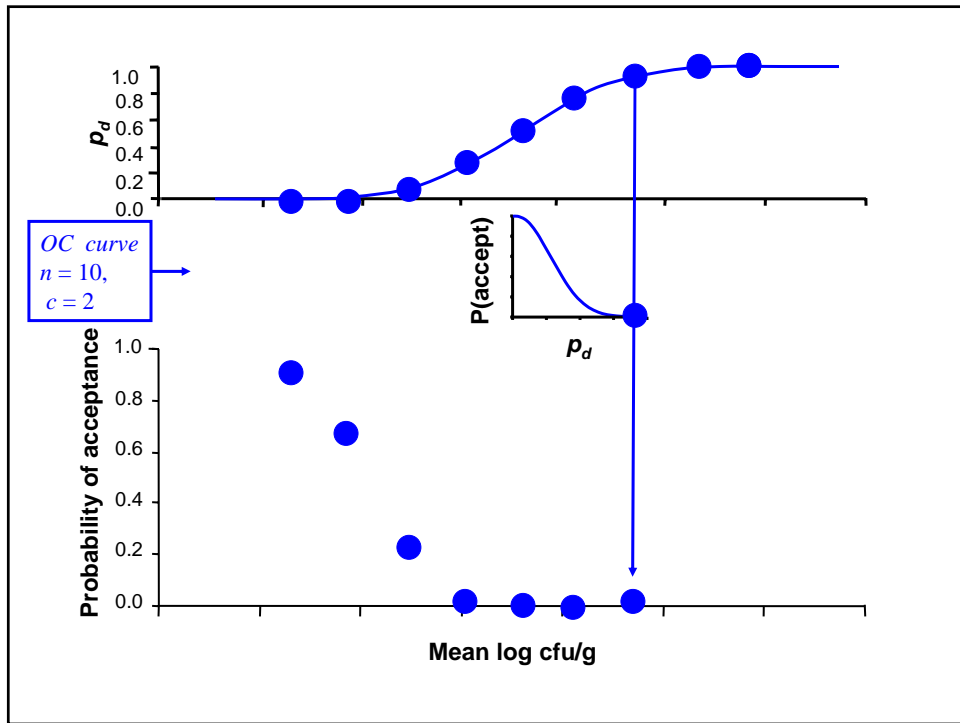
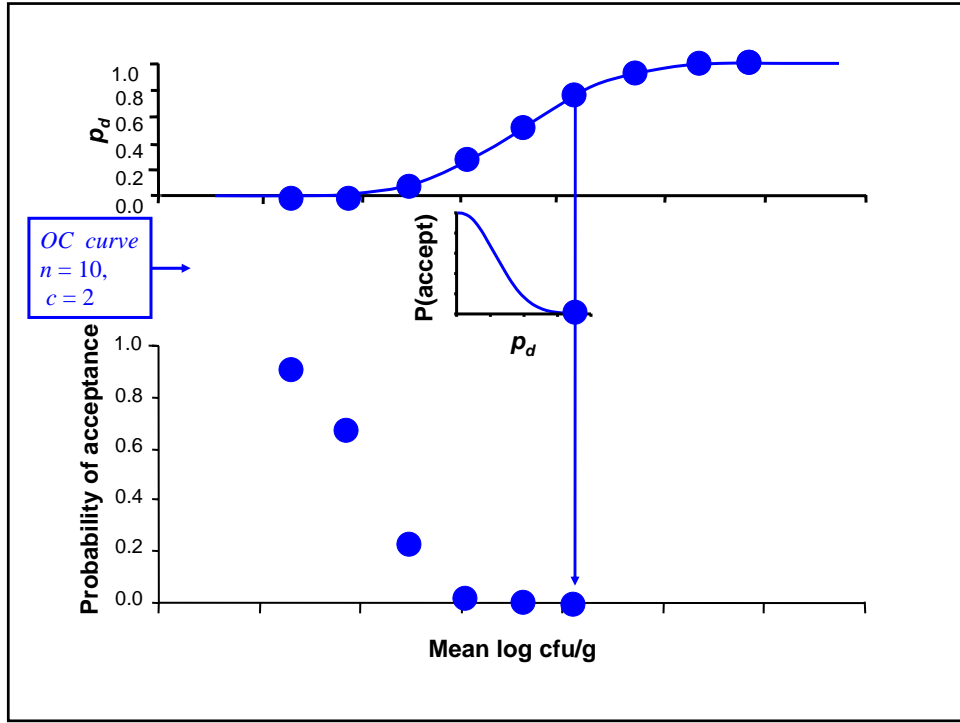


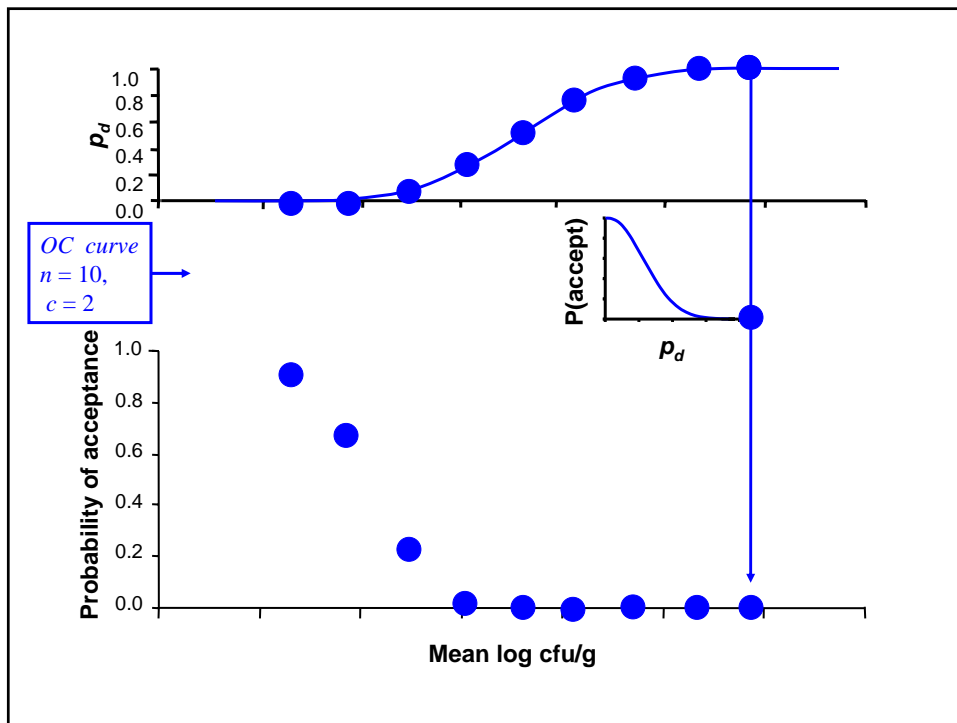
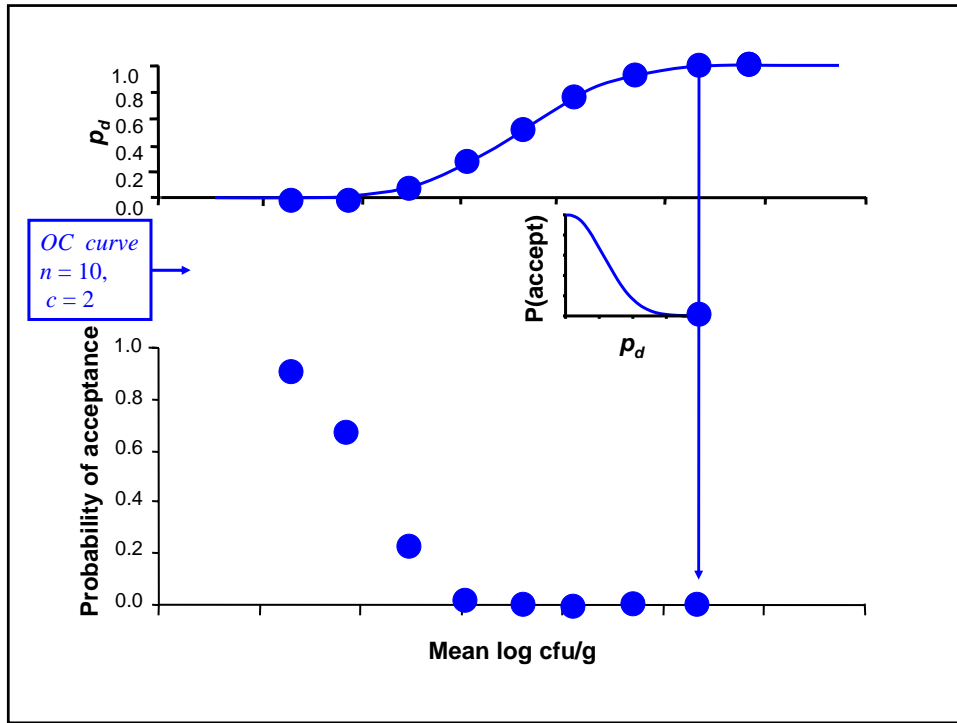


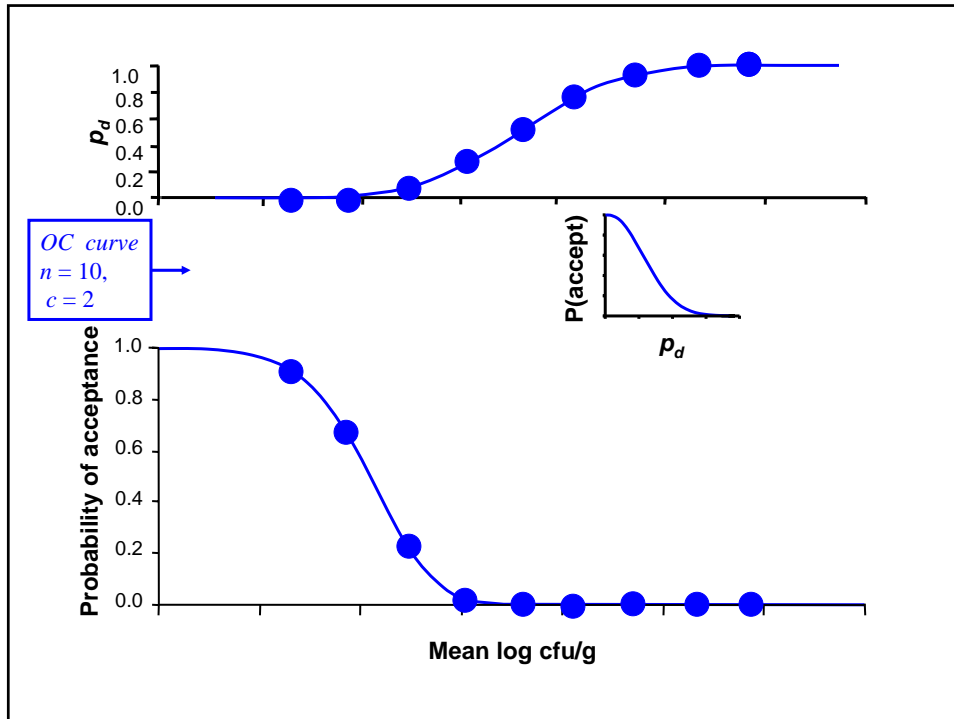










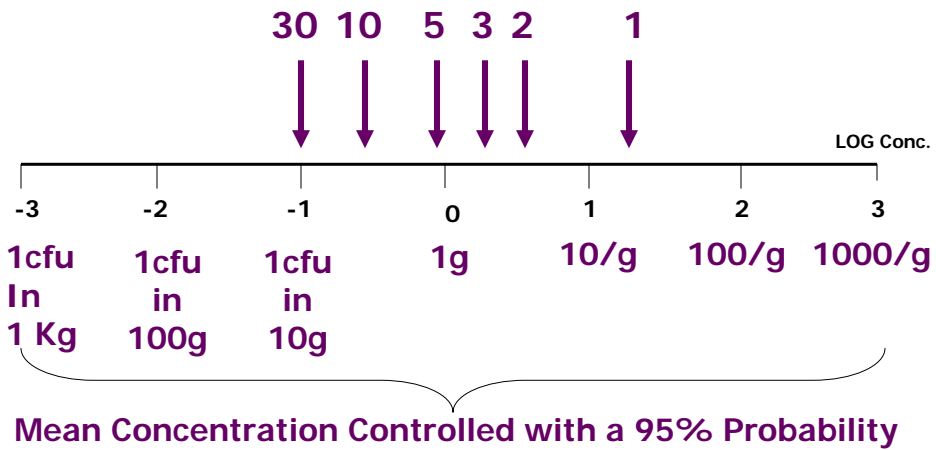


Performance of Sampling Plans

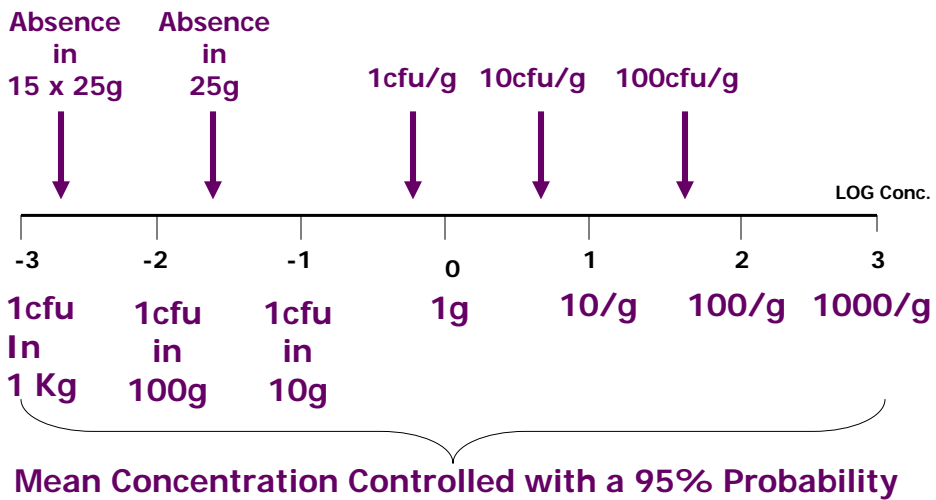
Sampling plan stringency, steepness of OC curve, location of critical lot qualities (95% probability of rejection, 95% probability of acceptance) depend on

- Plan specifications n and c
- Microbiological limits m and M
- Standard deviation s.d.
- Difference $M - m$ in relation to s.d.

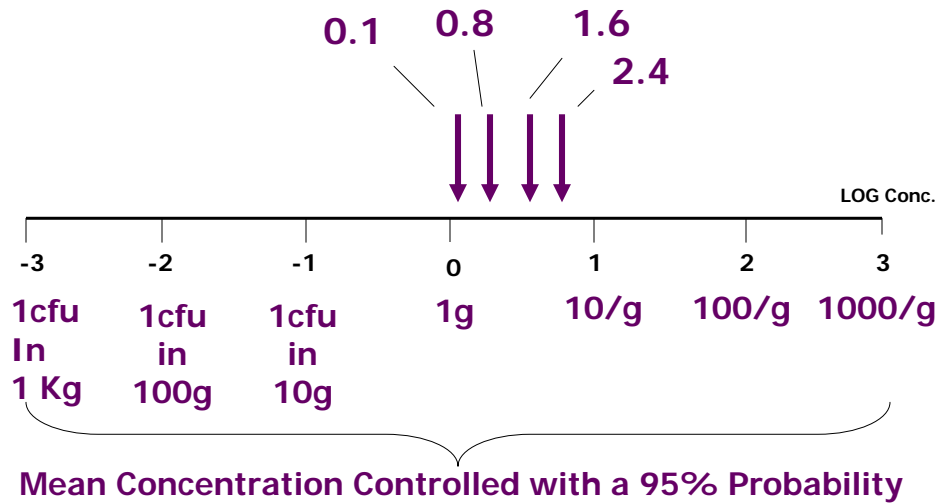
Effect of number of samples ($m=1/g$, $s.d. =0.8$)



Effect of limit m/sample size ($n=5$, $s.d. =0.8$)



Effect of Standard Deviation ($n=3, m = 1/g$)



ICMSF Three-Class Plans: Mean CFU/G Rejected With 95% Probability

Case 4: $n=5, c=3$ 5128 cfu/g	Case 5: $n=5, c=2$ 3311 cfu/g	Case 6: $n=5, c=1$ 1819 cfu/g
Case 7: $n=5, c=3$ 3311 cfu/g	Case 8: $n=5, c=1$ 1819 cfu/g	Case 9: $n=10, c=1$ 575 cfu/g

With:

$m = 1000$ cfu/g, $M = 10\,000$ cfu/g,
and standard deviation s.d. = 0.8

ICMSF Three-Class Plans: Mean Implied POs(based on mean count + 3sds)

Case 4: n=5, c=3 1288095 cfu/g	Case 5: n=5, c=2 881923 cfu/g	Case 6: n=5, c=1 456912 cfu/g
Case 7: n=5, c=3 831685 cfu/g	Case 8: n=5, c=1 456912 cfu/g	Case 9: n=10, c=1 144435 cfu/g

With:

$m = 1000$ cfu/g, $M = 10\ 000$ cfu/g,
and standard deviation s.d. = 0.8

ICMSF Two-Class Plans: Mean CFU/G Rejected With 95% Probability

Case 10: n=5, c=0 1 cfu / 32g	Case 11: n=10, c=0 1 cfu / 83g	Case 12: n=20, c=0 1 cfu / 185g
Case 13: n=15, c=0 1 cfu / 135g	Case 14: n=30, c=0 1 cfu / 278g	Case 15: n=60, c=0 1 cfu / 526g

With:

$m = 0$ cfu / 25g,
and standard deviation s.d. = 0.8

ICMSF Two-Class Plans: Implied PO (based on mean count +3sds)

Case 10: n=5, c=0 7.7cfu/g	Case 11: n=10, c=0 2.5cfu/g	Case 12: n=20, c=0 1.25cfu/g
Case 13: n=15, c=0 1.85cfu/g	Case 14: n=30, c=0 0.9cfu/g	Case 15: n=60, c=0 0.4cfu/g

With:
m = 0 cfu / 25g,
and standard deviation s.d. = 0.8

Relationship of Microbiological Criteria to FSO's

- **FSO:** A statement of the maximum frequency and/or concentration of a microbiological hazard in a food considered tolerable for consumer protection
- **Microbiological Criteria:** The acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms, and/or of mass, volume, area, or lot .

Summary and Conclusions

- No feasible sampling plan can ensure absence of a pathogen
- Microbial sampling is a 'Bernoulli trial'
- Stringency depends on key parameters
- Possible to link performance to mean concentration controlled

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Resources

ICMSF Sampling Plan Spreadsheet
www.icmsf.org