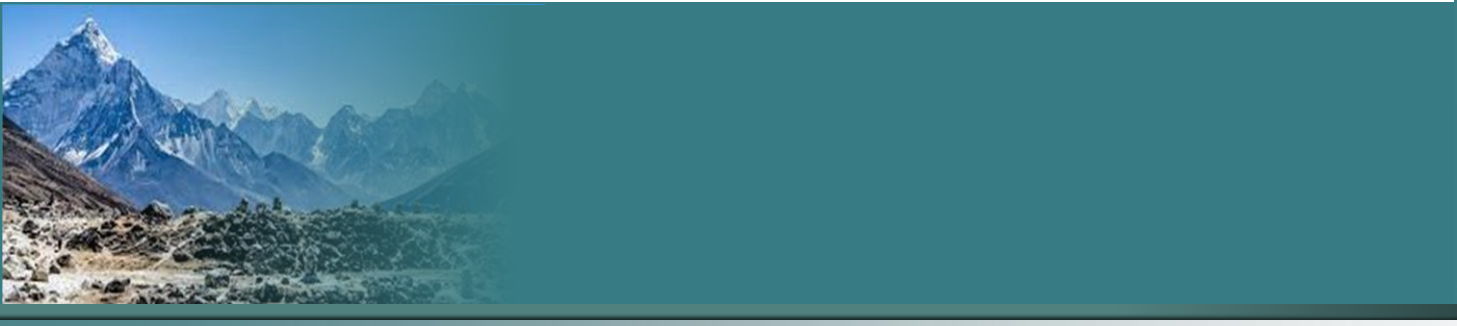
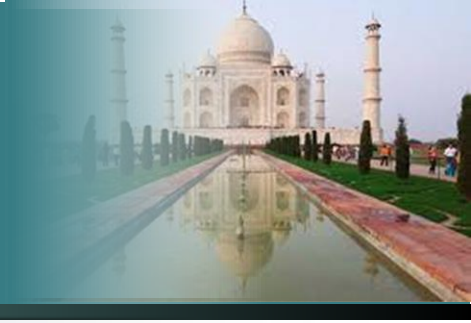




Use and Interpretation of the ICMSF Sampling Plan Tool

Tom Ross

Food Safety Centre, University of Tasmania, Australia

International Commission on Microbiological Specifications for Foods

Food Safety and Standards Authority of India

CHIFSS CII-HUL Initiative on Food Safety Sciences

tia Tasmanian Institute of Agriculture

Useful Reading

van Schothorst, M., Zwietering, M.H., Ross, T., Buchanan, R.L and Cole, M.B. International Commission on Microbiological Specifications for Foods. (2009). Relating microbiological criteria to food safety objectives and performance objectives. *Food Control,* **20**: 967-979.ICMSF

R.C. Whiting, A. Rainosek, R.L. Buchanan, M. Milioti, D. LaBarre, W. Long, A. Ruple and S. Schaub. (2006). Determining the microbiological criteria for lot rejection from the performance objective or food safety objective. *International Journal of Food Microbiology*, **110:** 263–267.

Useful Reading
(*continued*)

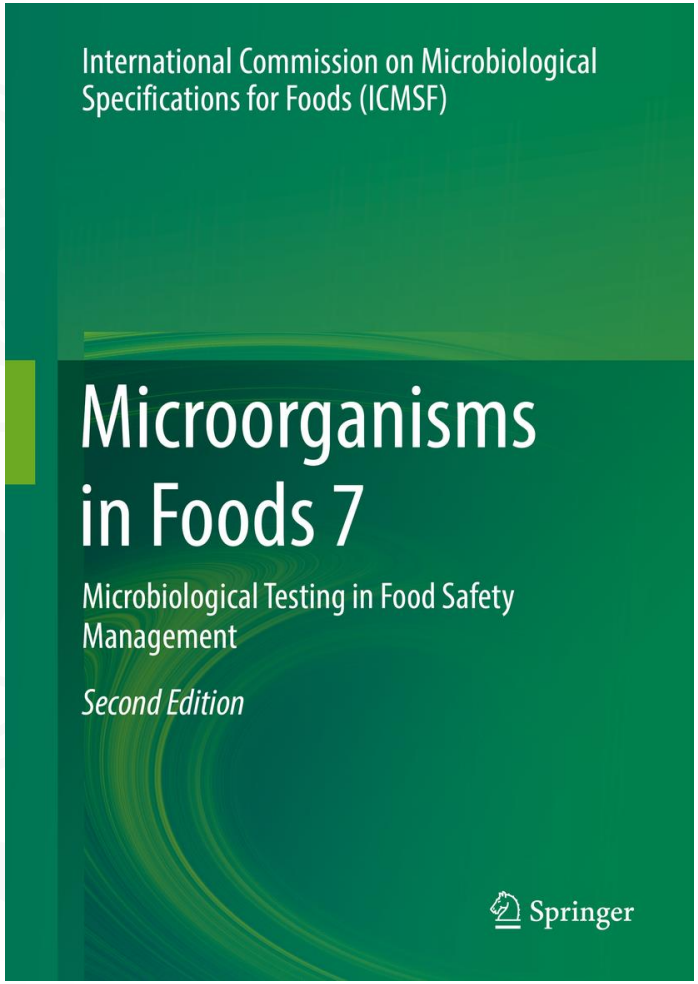
International Commission on Microbiological Specifications for Foods (ICMSF).

Microorganisms in Foods, Microbiological Testing in Food Safety Management:

Book 7, 2nd Edition (2018), 479pp.

Springer International Publishing

ISBN: 978-3-319-68458-1



Overview

- testing for compliance to a specified microbiological level:
  - implications of variability, sample size, sample number
- anatomy of the ICSMF sampling plan spreadsheet
- using the ICMSF spreadsheet to design a sampling plan to meet a criterion
- using the ICMSF spreadsheet to calculate the sensitivity of a sampling plan

Use and Interpretation of the ICMSF Sampling Plan Tool

the implications of variability

Designing an Appropriate Sampling Plan to Meet a Performance Objective

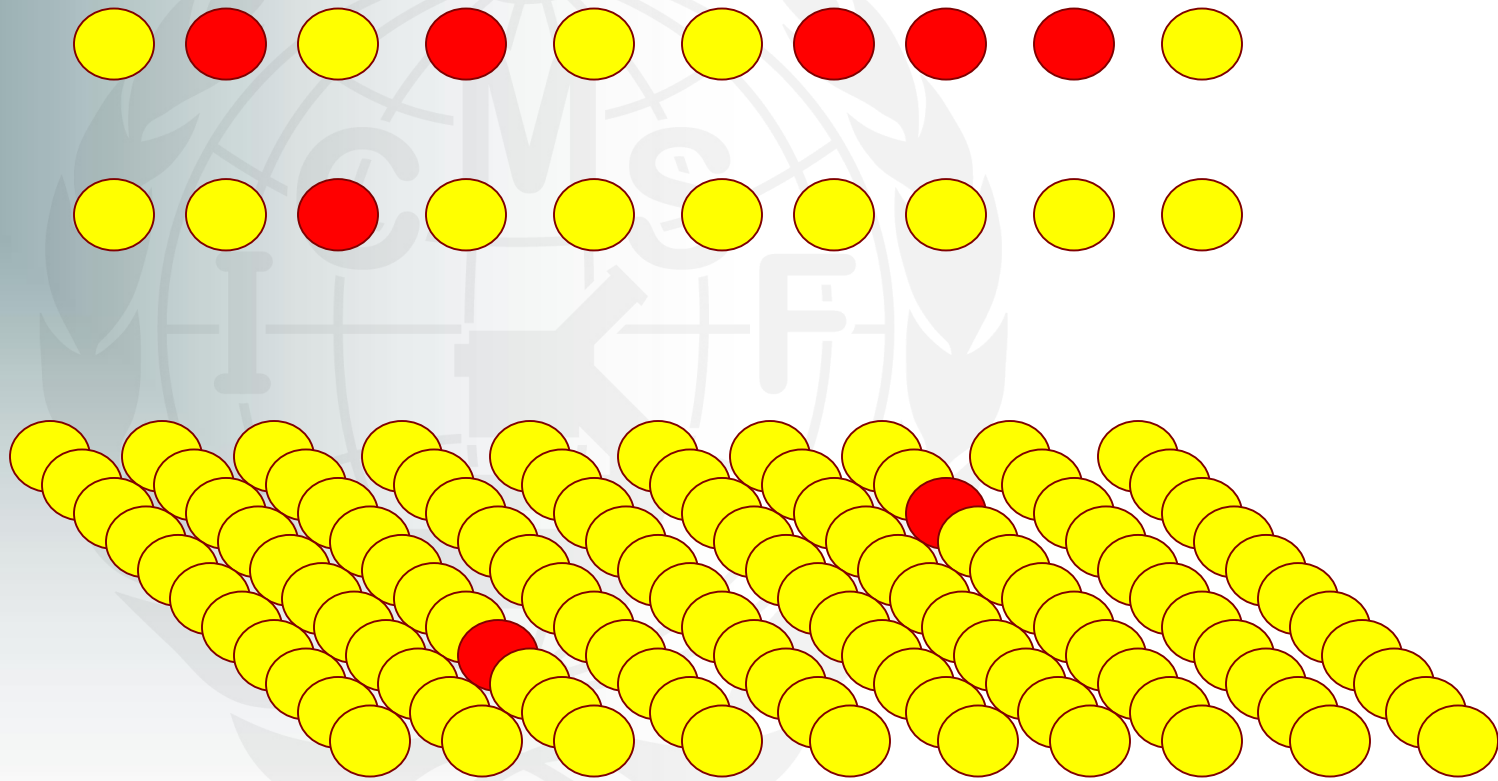
- Steps in the development of a sampling plan…
- For the specified standard:
  - define the '*just unacceptable lot*' from:
    - the standard deviation of contamination of samples within a lot
    - the required level of confidence
  - determine the needed performance of the analytical procedure (probability of false positives and false negatives)
  - determine the number of samples/sample size

Sampling and the Probability of Detection

<50% defective?

<10% defective?

<1 % defective?



Sampling and the Probability of Detection

we expect less than 0.01% contamination….

Sampling Plans: Probability of Detection

- sampling schemes try to identify contaminated batches of food by seeing what proportion are 'unacceptable'
- can't sample all units in the batch
- probability theory shows that the probability (P_{accept}) of not detecting contamination in a batch, by testing 'n' samples, when p is the true proportion of contaminated samples is:

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

Sampling Plans: Probability of Detection

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

P_{accept} = 0.05 (required confidence that the batch isn't contaminated)

p = 0.0001 (maximum tolerable proportion of contamination)

Want to solve the above for n, the number of samples that need to be tested to be sure that less than 1 in 10,000 is contaminated …

Sampling Plans: Probability of Detection

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

$$\log(P_{accept}) = \log((1-p)^n)$$

$$\log(P_{accept}) = n \times \log(1-p)$$

$$n = \log(P_{accept}) / \log(1-p)$$

$$= \log(0.05) / \log(0.999)$$

$$= 29956!$$

Testing: the importance of variability

if the contamination *within the lot* is always the same concentration, then acceptability can be determined by a single determination (test) because it is completely representative of the lot, and …

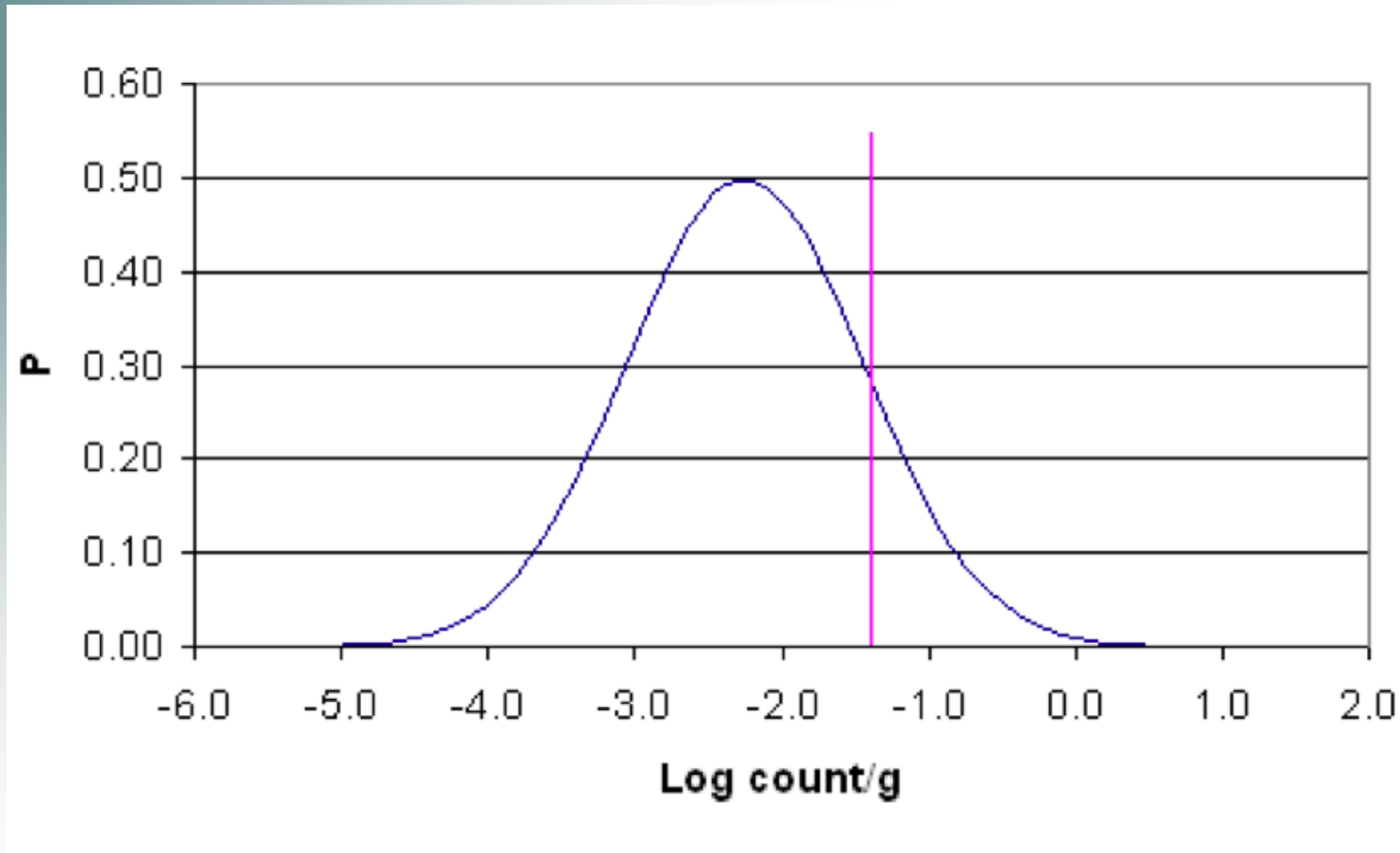
as long as the test result is less than the criterion, the lot is acceptable

Testing: the importance of variability

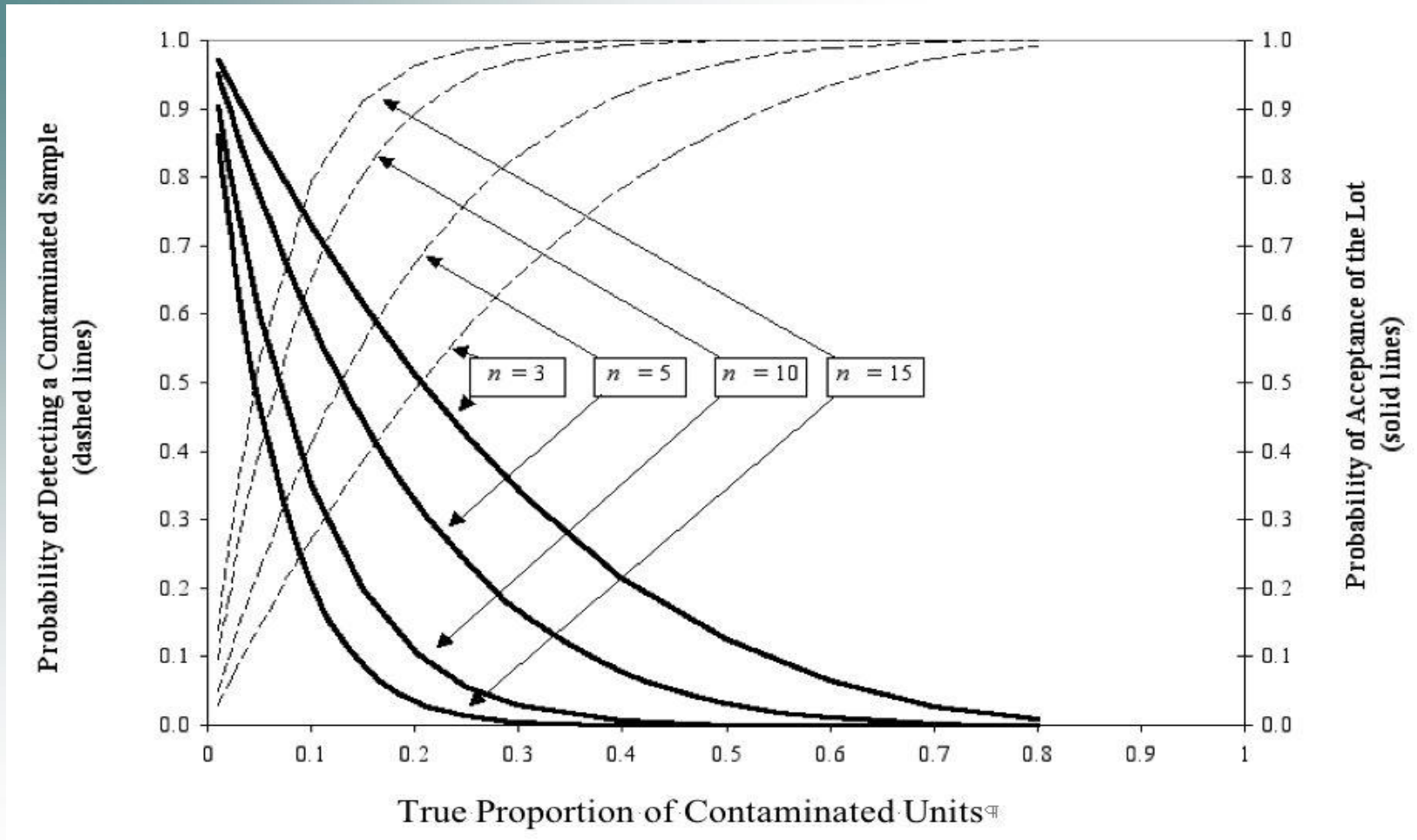
- usually, the contamination is not homogenous *within the lot* but is a distribution, characterised by a mean (‘average’) and standard deviation

- we need to work out *the mean concentration, so that the proportion of samples above the criterion is acceptably low* (*i.e.,* meets our specified confidence limit)

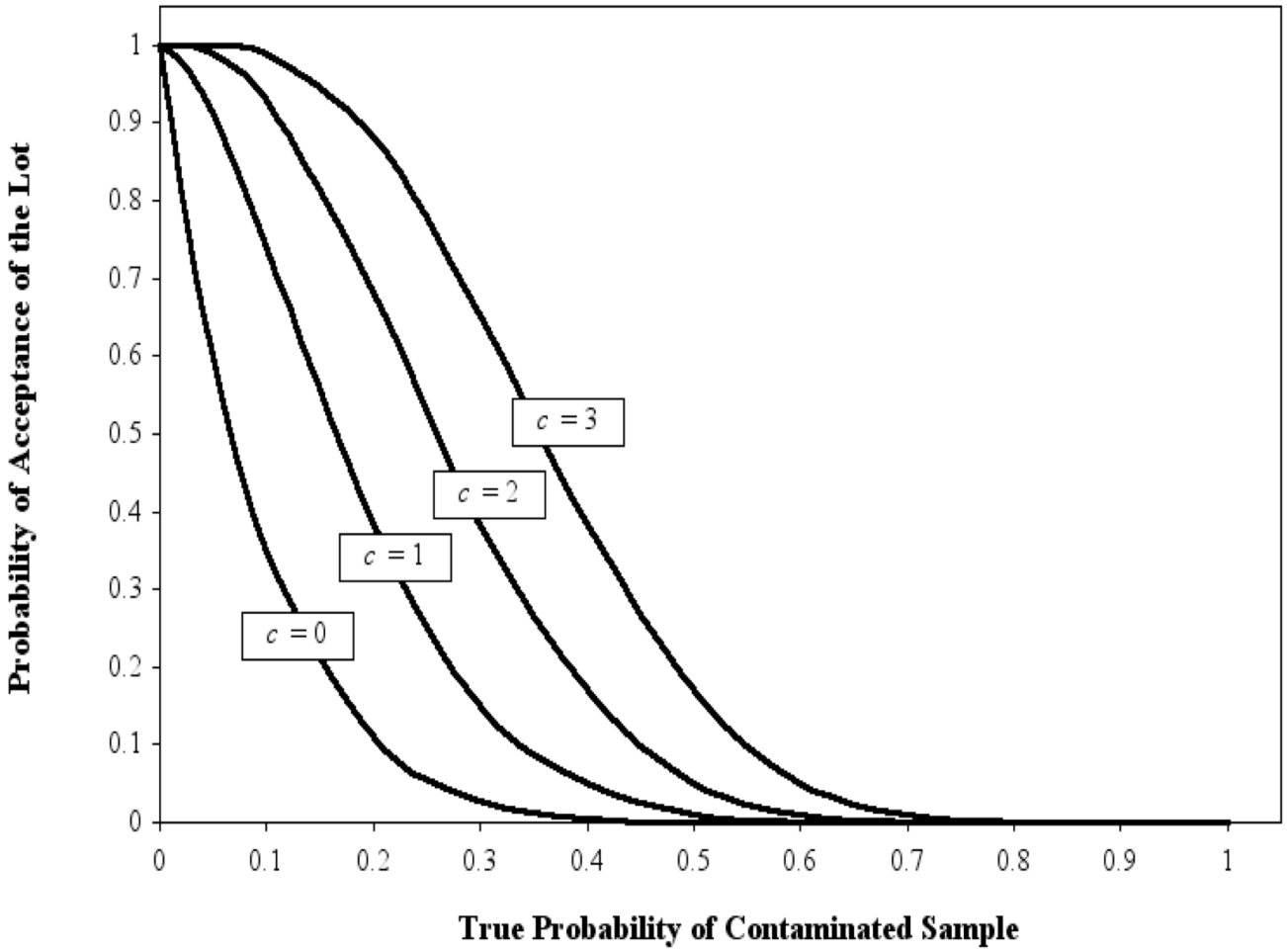
Use and Interpretation of the ICMSF Sampling Plan Tool



Effect of sample number, *n*, on plan stringency



Effect of *c* on plan stringency (*n* = 10)



Use and Interpretation of the ICMSF Sampling Plan Tool

the ICMSF spreadsheet….

http://www.icmsf.org/publications/software-downloads/

SOFTWARE DOWNLOADS

Microbiological sampling plans is a tool to explore ICMSF recommendations.

Standard Program

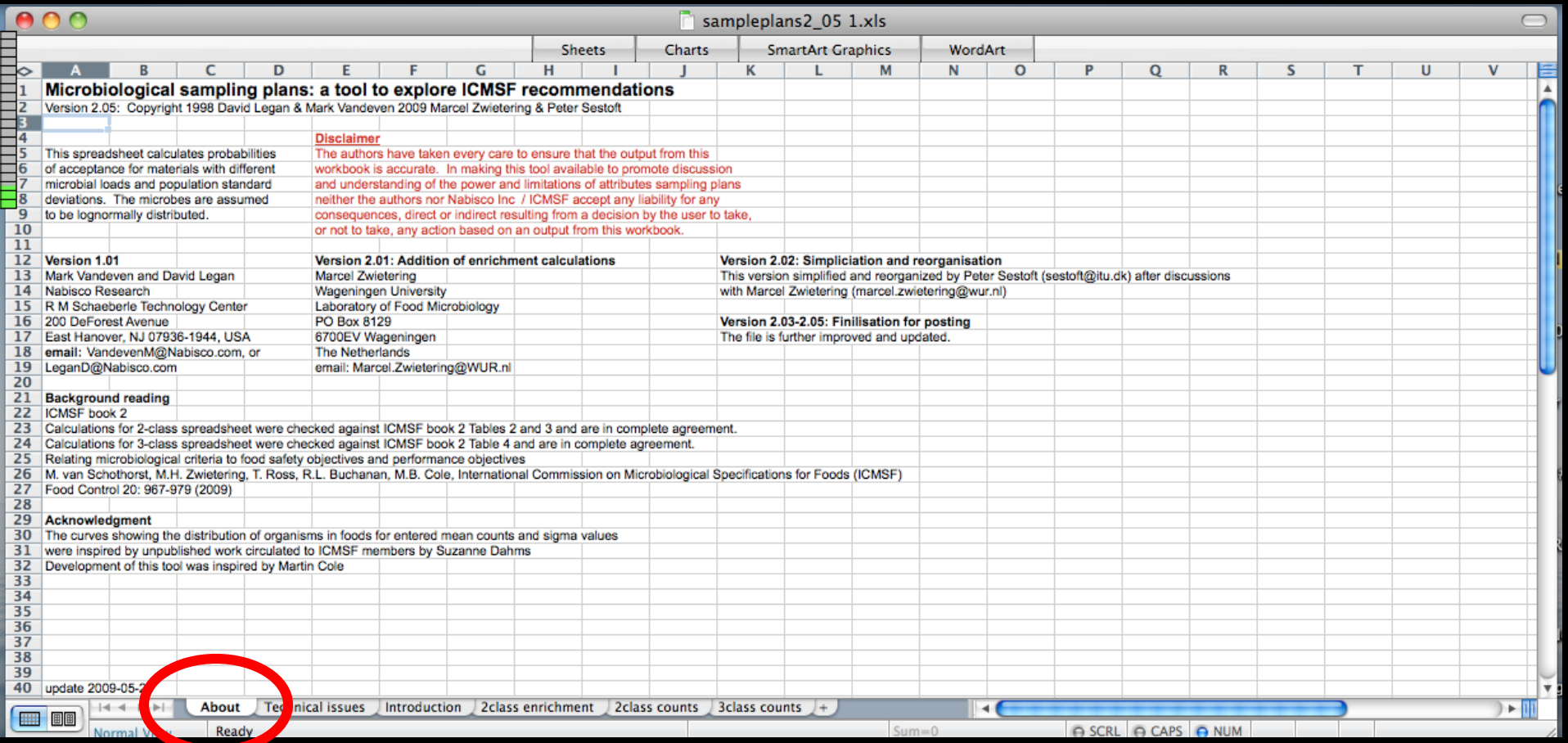
This spreadsheet calculates probabilities of acceptance for materials with different microbial loads and population standard deviations. The microbes are assumed to be lognormally distributed. This is new version 8 (November 2016) including additionally a tab with the effect of specificity and sensitivity.

Download (Spreadsheet 428 KB)

Control Measures Validation (FSO) Tool

A spreadsheet tool to explore the ICMSF Food Safety Objective (FSO) equation to determine the per cent compliance of products from processes that are affected by variability, and which is described in the publication "Validation of control measures in a food chain using the FSO concept (PDF 309KB)".

Download (Spreadsheet 171 KB)


sampleplans2_05 1.xls
Sheets
Charts
SmartArt Graphics
WordArt
Microbiological sampling plans: a tool to explore ICMSF recommendations
Version 2.05: Copyright 1998 David Legan & Mark Vandeven 2009 Marcel Zwietering & Peter Sestoft
This spreadsheet calculates probabilities of acceptance for materials with different microbial loads and population standard deviations. The microbes are assumed to be lognormally distributed.
Disclaimer
The authors have taken every care to ensure that the output from this workbook is accurate. In making this tool available to promote discussion and understanding of the power and limitations of attributes sampling plans neither the authors nor Nabisco Inc / ICMSF accept any liability for any consequences, direct or indirect resulting from a decision by the user to take, or not to take, any action based on an output from this workbook.
Version 1.01
Mark Vandeven and David Legan
Nabisco Research
R M Schaeberle Technology Center
200 DeForest Avenue
East Hanover, NJ 07936-1944, USA
email: VandevenM@Nabisco.com, or
LeganD@Nabisco.com
Version 2.01: Addition of enrichment calculations
Marcel Zwietering
Wageningen University
Laboratory of Food Microbiology
PO Box 8129
6700EV Wageningen
The Netherlands
email: Marcel.Zwietering@WUR.nl
Version 2.02: Simpliciation and reorganisation
This version simplified and reorganized by Peter Sestoft (sestoft@itu.dk) after discussions with Marcel Zwietering (marcel.zwietering@wur.nl)
Version 2.03-2.05: Finilisation for posting
The file is further improved and updated.
Background reading
ICMSF book 2
Calculations for 2-class spreadsheet were checked against ICMSF book 2 Tables 2 and 3 and are in complete agreement.
Calculations for 3-class spreadsheet were checked against ICMSF book 2 Table 4 and are in complete agreement.
Relating microbiological criteria to food safety objectives and performance objectives
M. van Schothorst, M.H. Zwietering, T. Ross, R.L. Buchanan, M.B. Cole, International Commission on Microbiological Specifications for Foods (ICMSF)
Food Control 20: 967-979 (2009)
Acknowledgment
The curves showing the distribution of organisms in foods for entered mean counts and sigma values were inspired by unpublished work circulated to ICMSF members by Suzanne Dahms
Development of this tool was inspired by Martin Cole
update 2009-05-2
About
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2class counts
3class counts
Ready
SCRL
CAPS
NUM

sampleplans2_05 1.xls

Working with the ICMSF sampling tool

Downloading the spreadsheet and technical issues

1) Download the ICMSF sampling tool by opening the web page:
http://www.icmsf.iit.edu/main/home.html
2) Select Download Standard Program in the right part of the screen, labelled as
"Download Standard Program" (sampleplans2_05.xls, 0.3M)
3) Respond to the question "open" or "save" the file by choosing "save" and save the file in an appropriate location.
4) Open this Excel file (sampleplans2_05.xls)
5) If you get a security warning concerning macros, please press "enable macros". (The macros are not viruses: instead they add useful functions to the spreadsheet).
For excel 2007 you have to enable macros in boxes top left of the screen
6) Select the tab "2class enrichment"
7) As opened cell M19 in the spreadsheet most probably reads 5%. Change cell J19 to -2. Cell M19 is now automatically recalculated and for this reason is no longer 5%. Click on the button "Find mean that gives…" in the grey box below cell M20. If you do not get a security warning and if Cell M19 changes to 5%, the file is working correctly and you can go on at the examples in the Introduction (other worksheet).
If you *do* get a security warning, or if cell M19 does not change to 5%, your system is not yet working correctly with the software and you should consider the following:

8) FOR PC/Windows USERS:
If macros are not enabled:
Go to the "***Tools***" pull-down menu, select "macros" "security" and set security level at "medium", read the file again and continue at 5, now it should work.

9) FOR Macintosh USERS:

For Excel users, the macros (linked to 'buttons') will probably not work as intended. Users of Excel 2004 for Macintosh may find that the buttons work, but they will probably not operate as intended in Excel X or Excel 2008. Other functions will operate normally, however, and many calculations can still be performed.

For Users with recent Macintosh computers

If you want to use the full capability of the spreadsheet, it will be better if you can use Excel 2004 (part of *Mac for Office, 2004*) or set your computer up to run a PC emulation. Various software are available to do this, e.g. "Parallels" (http://www.parallels.com). You can run PC emulation software effectively on an Intel-based Macintosh. You may need to install Windows operating system, and a PC version of MS Excel. Once that is done, follow the instructions above.

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Description of the used variables

Data entry boxes are highlighted in yellow: All other cells are protected.

mean	mean of the (assumed) log normal distribution describing the occurrence of bacterial contaminants; unit is log cfu per gram
sigma	standard deviation of the same (assumed) log normal distribution
m	acceptable level of microbiological contamination, defined as an allowable concentration, or for enrichement no contamination in a sample of a certain weight, her
n	number of samples tested
c	number of samples whose contamination is allowed to exceed m (that is, test positive for contamination), yet the lot will be accepted
amount	sample weight, in gram
P(accept)	the probability of accepting a specific lot; this is a function of the assumed contamination level (mean, sigma) and the sampling plan (n, c and amount)

For a manual please open the following word-file

D:\Marcel\icmsf\newtool\Working wit

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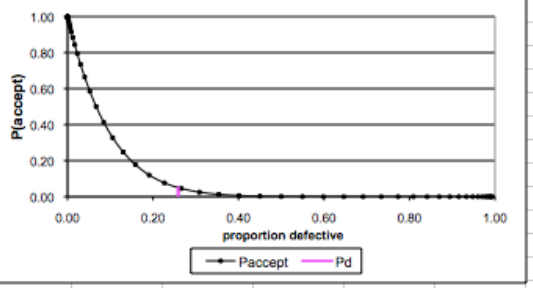
Normal View | Ready | Sum=0 | SCRL | CAPS | NUM

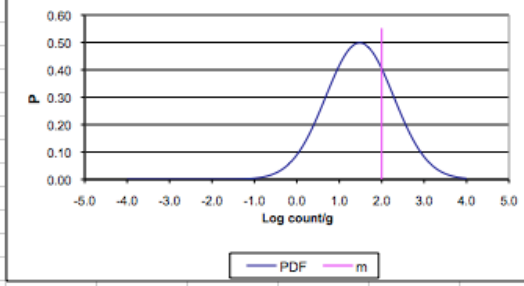
sampleplans2_05 1.xls

Sheets | Charts | SmartArt Graphics | WordArt

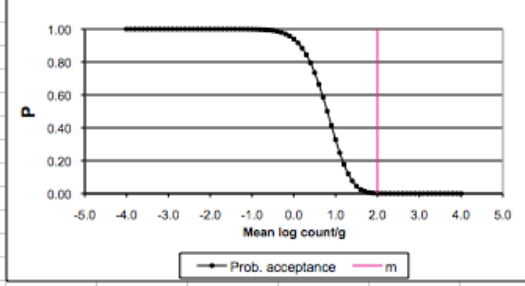
Operating characteristic curve for proportion defective, with n=10 and c=0



Probability density function (PDF) for log counts. Distribution mean = 1.48 and sigma = 0.80



Operating characteristic curve scaled to relate mean log count to m



Batch acceptance for Pd			P(accept)	
Pd	10	%	34.9	%
actualPd	25.9	%	5.00	%

INPUTS	
mean	1.48
sigma	0.80
m	2
n	10
c	0

P(accept)		
Computed	5.00	%
Desired	5	%

Find mean that gives desired

Find n that gives desired

ALTERNATIVE n AND c	
mean	1.48
sigma	0.80
m	2.53
n	30
c	0

P(accept)		
Computed	5.00	%
Target, left	5.00	%

For any value of n and c imputed find the m that

Sandbox: for your own calculations

Means and median			
Arithmetic		Geometric=median	
165.7	cfu/g	30.4	cfu/g

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	3.96

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Normal View | Ready | Sum=1.48 | SCRL | CAPS | NUM

sampleplans2_05 1.xls

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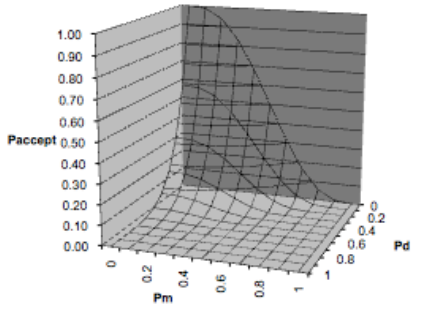
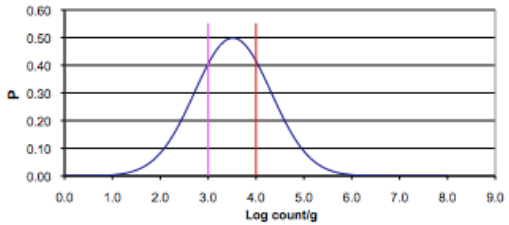
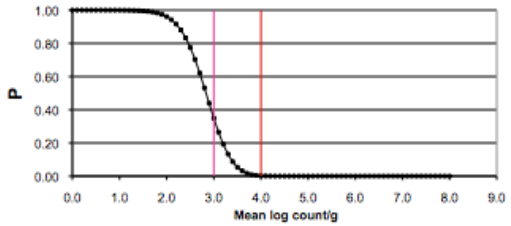
Operating characteristic curve for proportion defective Pd and marginally acceptable Pm with n=5 and c=2



Probability density function (PDF) for log counts. Distribution mean = 3.52 and sigma = 0.80



Operating characteristic curve scaled to relate mean log count to m



Batch acceptance for Pd			P(accept)	
Pd	20	%	26.9	%
Pm	25	%		
actualPd	27.3	%		
actualPm	46.8	%	5.00	%

Sandbox: for your own calculations

INPUTS	
mean	3.52
sigma	0.8
m	3
M	4
n	5
c	2

P(accept)		
Computed	5.00	%
Desired	5	%

Find mean that gives desired

Find n that gives desired

ALTERNATIVE n AND c	
mean	3.52
sigma	0.80
m	4.03
M	5.03
n	10
c	0

P(accept)		
Computed	5.00	%
Target, left	5.00	%

For any value of n and c imputed find the m that

Means and median			
Arithmetic		Geometric=median	
17903.6	cfu/g	3281.8	cfu/g

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	5.99

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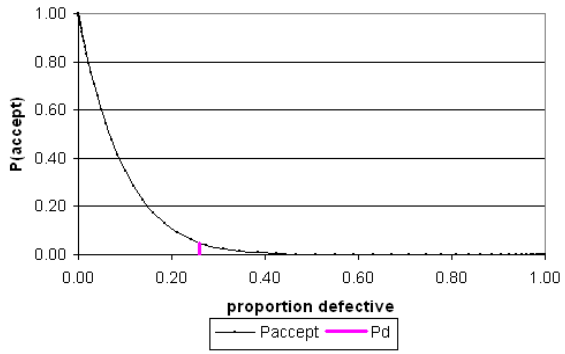
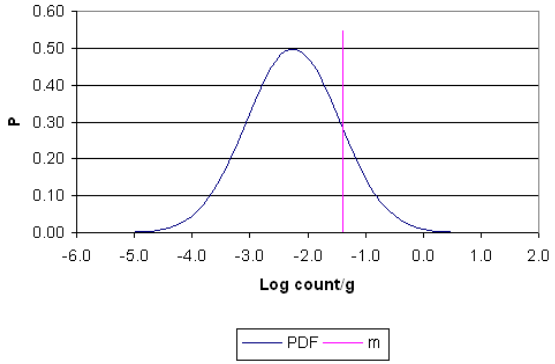
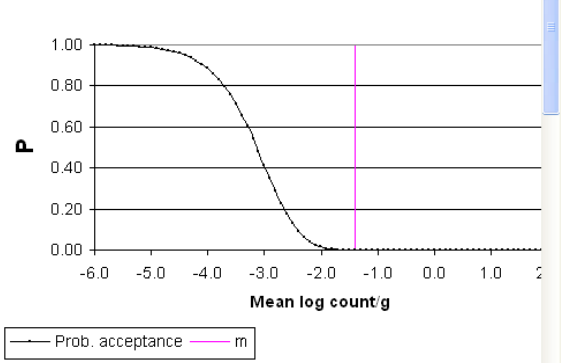
Normal View | Ready | Sum=3.52 | SCRL | CAPS | NUM

ICMSF Sampling plans spreadsheet

Microsoft Excel - sampleplans2_05.xls







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		Find mean that gives desired P(accept)		
c	0				
amount	25	g	Find n that gives desired P(accept) or better (less)		

ALTERNATIVE n AND c			P(accept)		
mean	-2.25		Computed	0.91	%
sigma	0.80		Target, left	5.00	%
m	-0.98				
n	30		For any value of n and c imputed find the m that gives the same P(accept) as the model on the left		
c	0				
amount	9.6	g			

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

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	0.23

The calculations below are for the buttons above. Do not edit.

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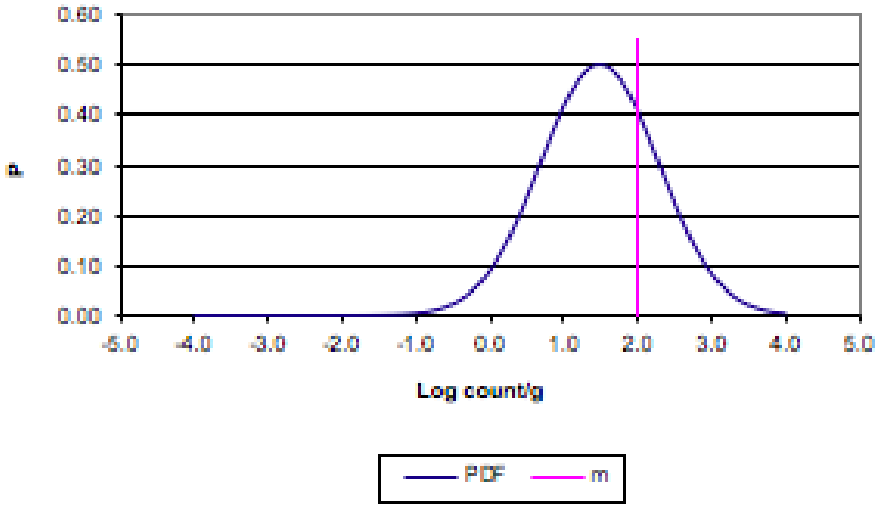
Elements of the spreadsheet

- **mean** = *required* average value of distribution (log_{10}CFU) of counts *in the lot*
- **sigma** = standard deviation known, or assumed (and assumed to be the same between lots)
- **m*** = detection threshold of test method (e.g. 2 log_{10}CFU; -1.4 log_{10}CFU; for presence/absence is log (inverse of sample size); often also the microbiological criterion
- **n** = number of samples tested
- **c** = number of samples permitted to fail the test
- $\mathbf{P_{accept}}$ = confidence required (or achieved) in the reliability of the sampling plan
- *various 'buttons'*

Probability density function (PDF) for log counts. Distribution mean = 1.48 and sigma = 0.80



INPUTS	
mean	1.48
sigma	0.80
m	2
n	10
c	0

P(accept)		
Computed	5.00	%
Desired	5	%

Find mean that gives desired P(accept)

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

Preject	95.00

Means and median

Arithmetic		Geometric=median	
165.7	cfu/g	30.4	cfu/g
2.22	log cfu/g	1.48	log cfu/g

extra help …

Excel File Edit View Insert Format Tools Data Window Help

sampleplans2_05 1.xls

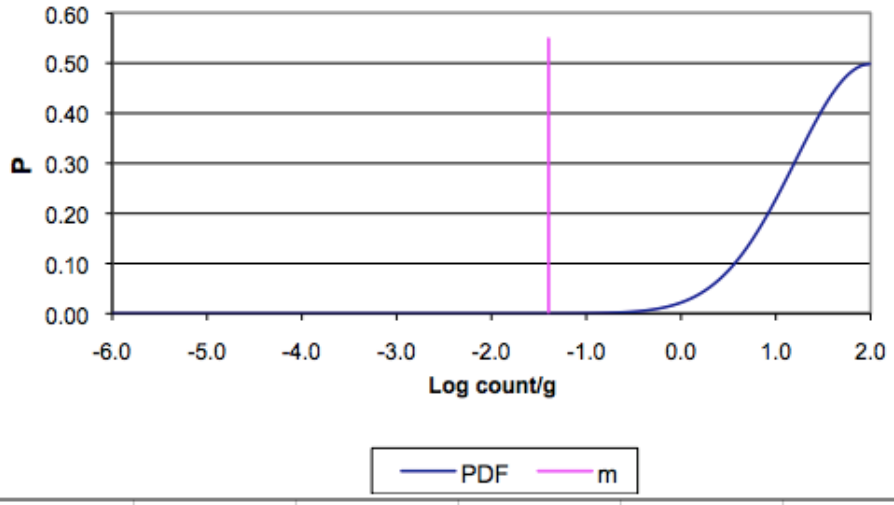
Sheets Charts SmartArt Graphics WordArt

rtion defective, with

0.80 1.00

d

Probability density function (PDF) for log counts.
Distribution mean = 2.00 and sigma = 0.80



P(accept)	
10.7	%
#NAME?	%

ations

INPUTS		
mean	2.00	
sigma	0.80	
m	-1.40	
n	10	
c	0	
amount	25	g

Mean log(10) count/g

The spreadsheet assumes that this is the mean of a normal distribution of log counts with standard deviation = sigma (cell J20)

The probability density function is calculated from this mean and the sigma below

Find n that gives desired

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

Normal View Cell I19 commented by David Legan Sum=0.80

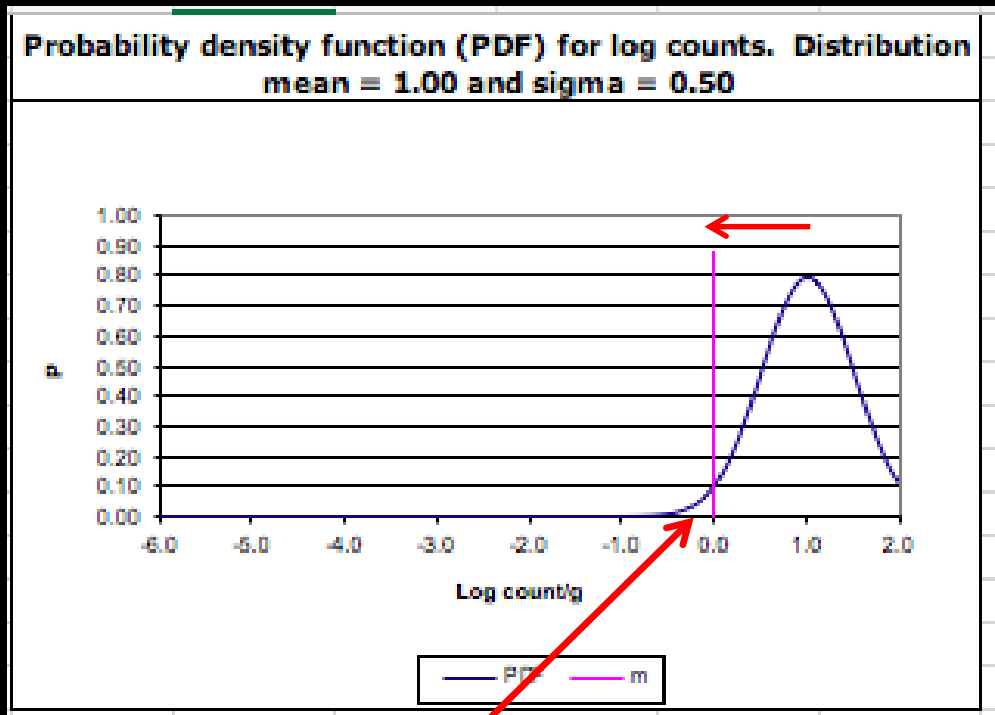
Designing an Appropriate Sampling Plan to Meet a Performance Objective

- Steps in the development of a sampling plan…
- For the specified criterion:
  - define the '*just unacceptable lot*' from:
    - the stated microbiological criterion
    - the standard deviation of contamination of samples within a lot
    - the required level of confidence
  - determine the required performance of the analytical procedure (probability of false positives and false negatives)
  - determine the number of samples/sample size

For criterion of 1 cfu/g ….

this distribution of contamination levels is unacceptable.

We could ….



INPUTS		
mean	1.00	
sigma	0.50	
m	0.00	
n	30	
c	0	
amount	1	g

P(accept)		
Computed	0.00	%
Desired	5	%

Find mean that gives desired P(accept)

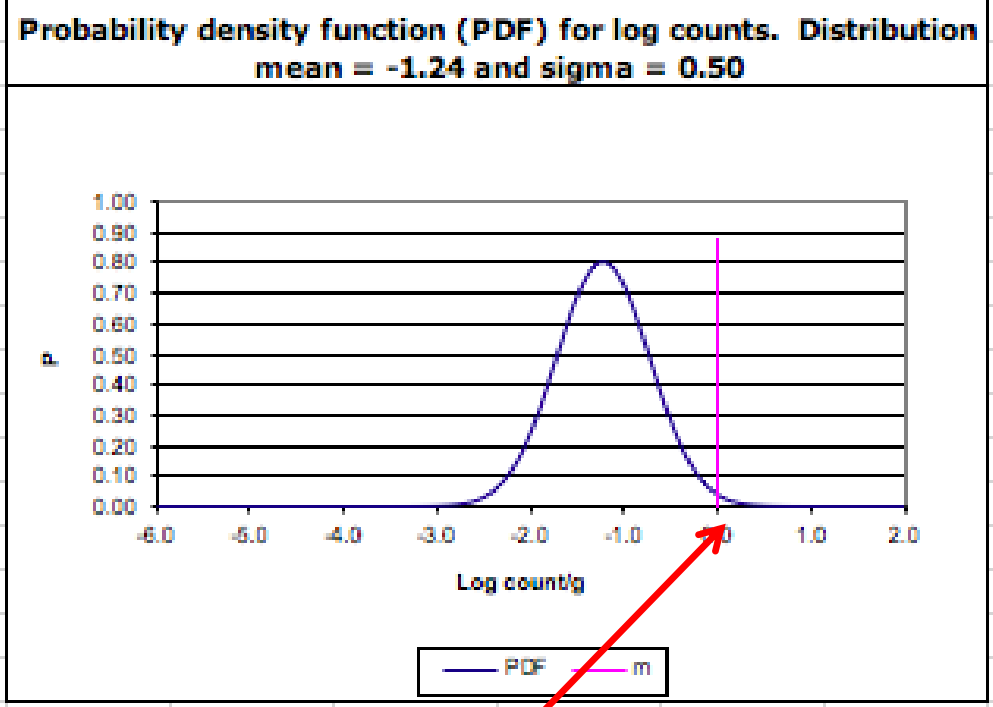
Find n that gives desired P(accept) or better (less)

Preject 100.00

Means and median					
	Arithmetic			Geometric=median	
	19.4010	cfu/g		10.0000	cfu/g
one cfu in	0.1	grams	one cfu in	0.1	grams
	1.29	log cfu/g		1.00	log cfu/g

… decrease the mean.

But the variation (standard deviation) also has an effect …..

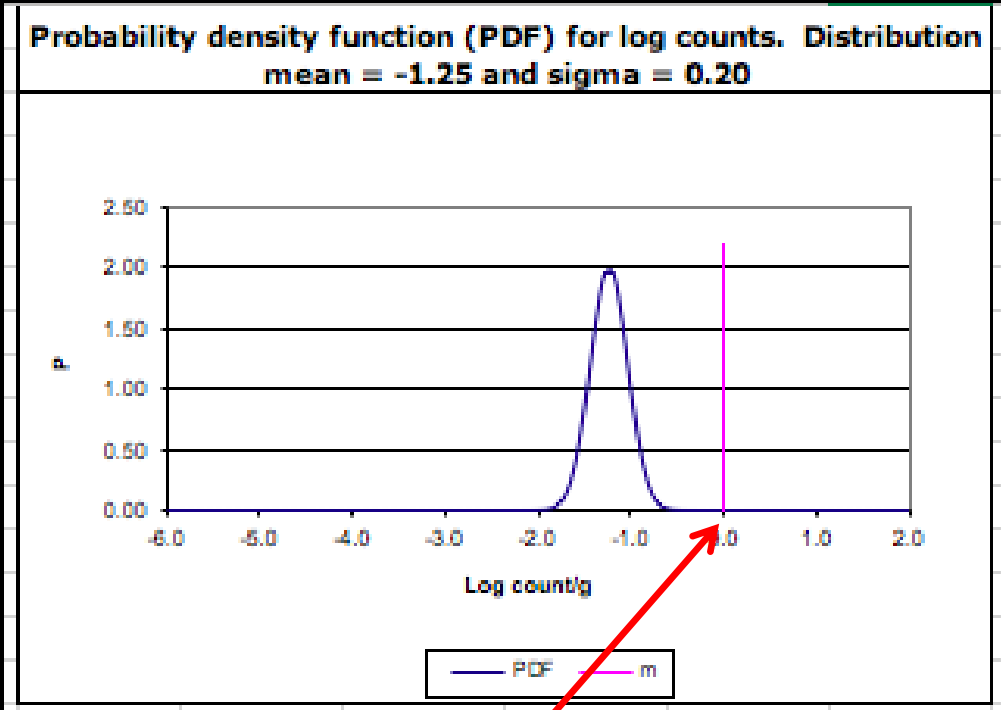


INPUTS			P(accept)		
mean	-1.24		Computed	5.00	%
sigma	0.50		Desired	5	%
m	5.00		Find mean that gives desired P(accept)		
n	30				
c	0		Find n that gives desired P(accept) or better (less)		
amount	1	g			
			Preject	95.00	

Means and median					
	Arithmetic			Geometric=median	
	0.1121	cfu/g		0.0578	cfu/g
one cfu in	8.9	grams	one cfu in	17.3	grams
	-0.95	log cfu/g		-1.24	log cfu/g

… do something to decrease the variation in counts.

In this case, the mean could be higher….



INPUTS		
mean	-1.25	
sigma	0.20	
m	0.00	
n	48	
c	0	
amount	1	g

P(accept)		
Computed	5.00	%
Desired	5	%

Find mean that gives desired P(accept)

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

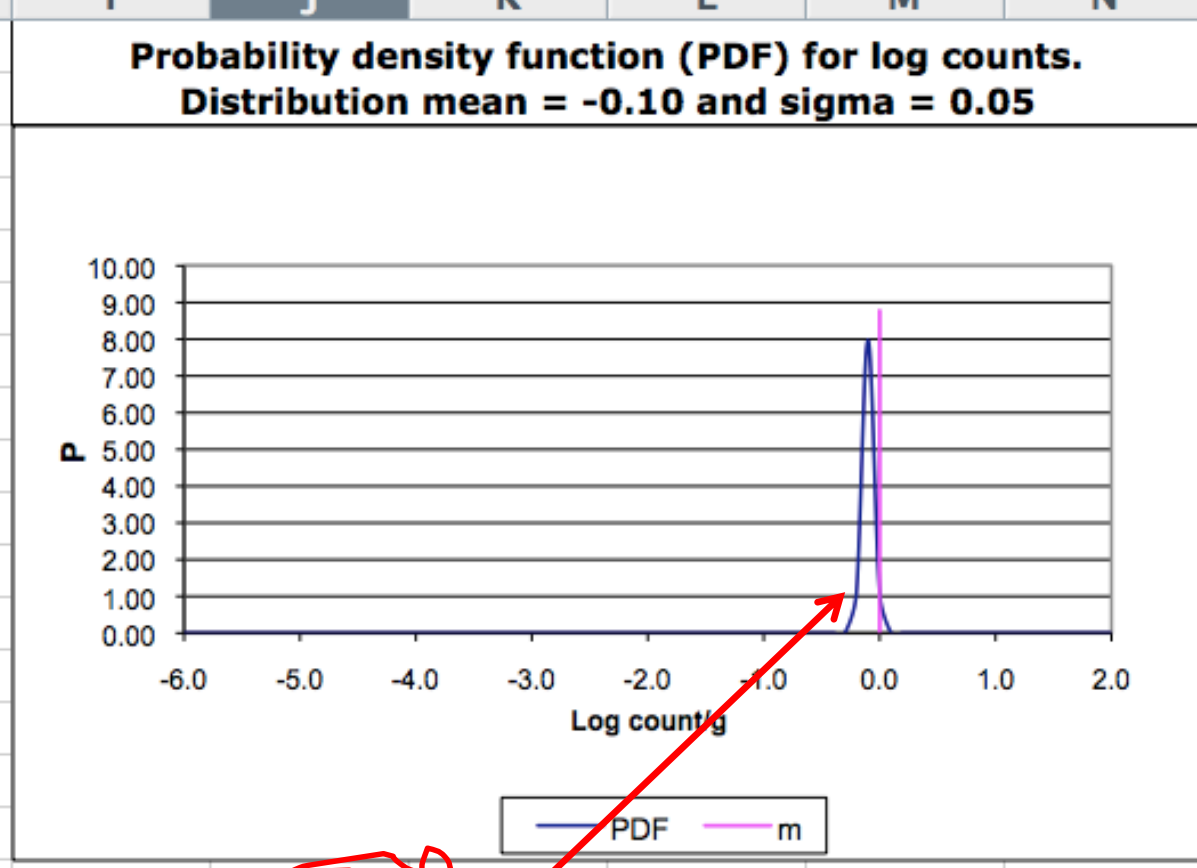
Preject 95.00

Means and median					
	Arithmetic			Geometric=median	
	0.0629	cfu/g		0.0565	cfu/g
one cfu in	15.9	grams	one cfu in	17.7	grams
	-1.20	log cfu/g		-1.25	log cfu/g

For criterion of 1 cfu/g ….

this distribution of contamination levels is acceptable.

Even though the mean is higher, the variation is less, and we can go “closer” to the criterion.



INPUTS		
mean	-0.10	
sigma	0.05	
m	0.00	
n	5	
c	0	
amount	1	g

P(accept)		
Computed	#NAME?	%
Desired	5	%

Find mean that gives desired

Find n that gives desired

Solver

- Calculates solution to "*what-if*" problems based on adjustable cells and constraint cells
- uses iterative ('searching') techniques to find an optimum value to solve an equation

Designing an Appropriate Sampling Plan to Meet a Performance Objective

- Steps in the development of a sampling plan…
- For the specified criterion:
  - define the '*just unacceptable lot*' from:
    - the stated microbiological criterion
    - the standard deviation of contamination of samples within a lot
    - the required level of confidence
  - determine the required performance of the analytical procedure (probability of false positives and false negatives)
  - determine the number of samples/sample size
  - Use the spreadsheet buttons to find the sampling required

Use and Interpretation of the ICMSF Sampling Plan Tool

sampling: binomial distributions and required levels of confidence

Designing an Appropriate Sampling Plan to Meet a Performance Objective

- binomial distributions
  - either we detect a microorganism(s) in the sample or we do not
- probability of detection depends
  - concentration in the lot
  - size of the sample
  - e.g. if the concentration is *1 per 10 g* we expect to detect ("presence") in most 25 g samples, but if the concentration is *1 per 50 g*, we expect to detect in only every second sample, "on average"

Designing an Appropriate Sampling Plan to Meet a Performance Objective

- often, when testing for pathogens, the acceptable mean concentration is small
- consequently, only a small proportion of samples to be tested are expected to be positive (have detectable pathogens)
- this means we are sampling from a Poisson process
- we need to know how many samples to take to be highly confident that the mean concentration is below our acceptable limit

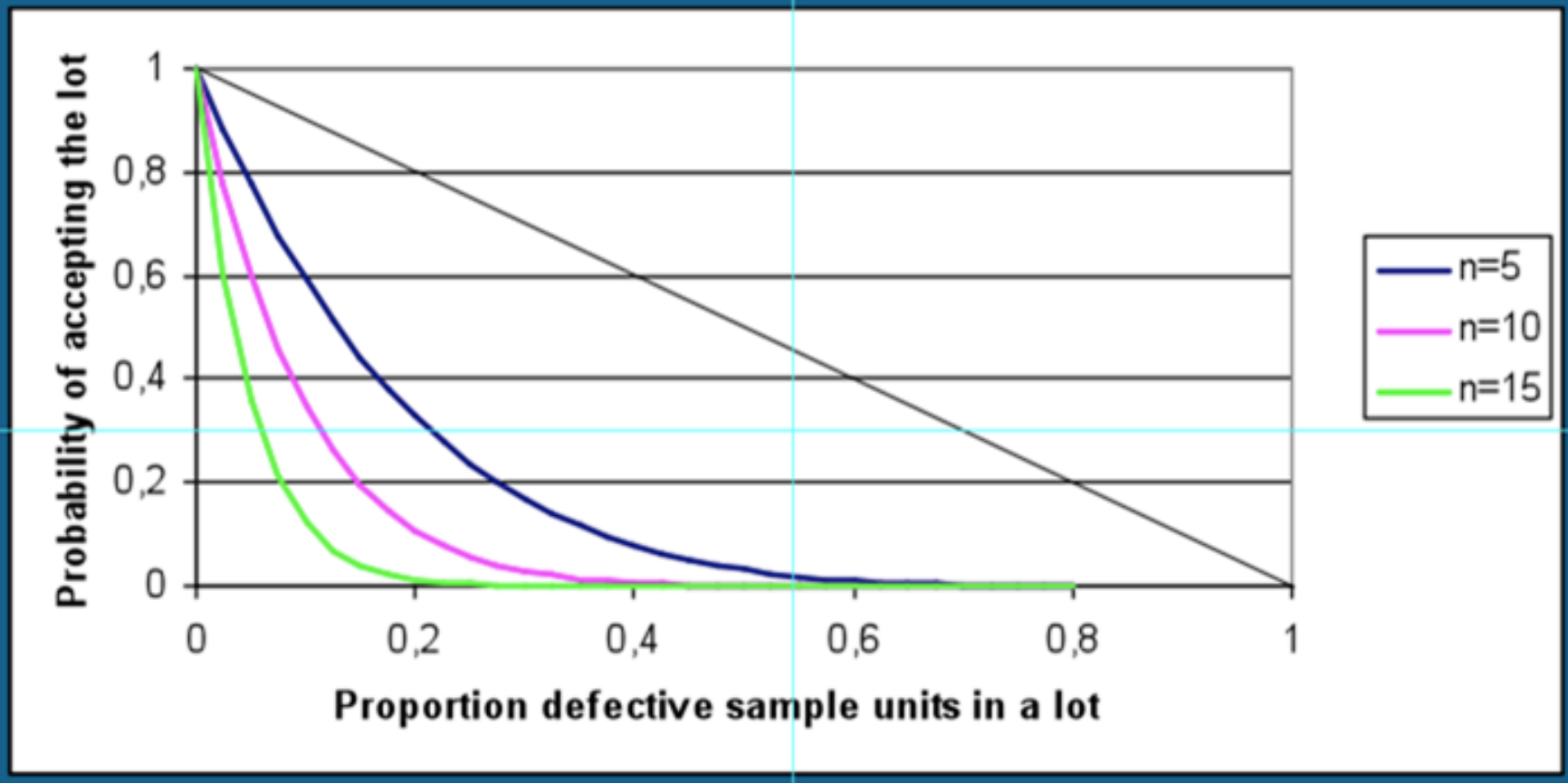
Probability that all samples are negative

P defective	P 1 x negative (1- Pdef) =	P 2 x negative (1- Pdef)^2 =	P 5 x negative (1- Pdef)^5 =
0.00	1.00	1.00	1.00
0.05	0.95	0.90	0.77
0.10	0.90	0.81	0.59
0.15	0.85	0.72	0.44
0.20	0.80	0.64	0.33
0.25	0.75	0.56	0.24
0.30	0.70	0.49	0.17

10.000 bars

0.9^60=0.0018

Probability of accepting a defective lot !



Designing an Appropriate Sampling Plan to Meet a Performance Objective

- Steps in the development of a sampling plan…
- For the specified standard:
  - define the *'just unacceptable lot'* from:
    - the standard deviation of contamination of samples within a lot
    - the required level of confidence
  - determine the needed performance of the analytical procedure (probability of false positives and false negatives; *e.g.,* determined from OC curves)
  - determine the number of samples/sample size

review of process

- what is the criterion
- what is the standard deviation in the lot
- what is the ‘tolerable’ rate of non-compliance
- what is the test method sensitivity (inverse of sample size for presence/absence)
- how many samples are needed (binomial sampling) to achieve the confidence required that the test result ‘correct’

Use and Interpretation of the ICMSF Sampling Plan Tool

Using the Spreadsheet to Estimate Sampling Plan 'Sensitivity'

Using the Spreadsheet to Estimate Sampling Plan 'Sensitivity'

- The performance of the sampling plan is affected by
  - the number of samples tested
  - the size of the samples tested (and detection limit of each sample test; bigger samples should give higher prevalence)
  - the standard deviation of the counts within the batch

ICMSF Sampling Plan Guidance

increasing probability

increasing severity

	Conditions expected after sampling		
	Reduction in Cell Density	No Change in Cell Density	Increase in Cell Density
Utility	Case 1	Case 2	Case 3
Indicator	Case 4	Case 5	Case 6
Moderate hazard	Case 7 (n=5, c=2)	Case 8 (n=5, c=1)	Case 9 (n=10, c=1)
Serious hazard	Case 10 (n=5, c=0)	Case 11 (n=10, c=0)	Case 12 (n=20, c=0)
Severe hazard	Case 13 (n=15, c=0)	Case 14 (n=20, c=0)	Case 15 (n=60, c=0)

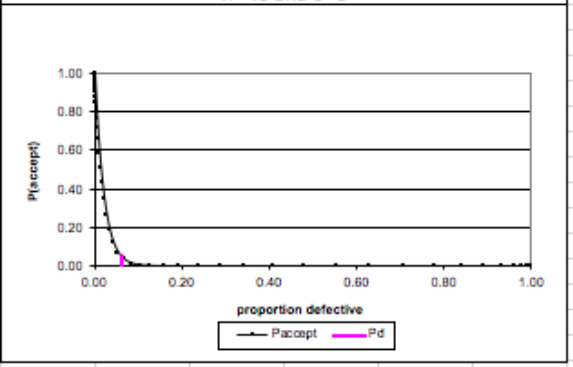
ICMSF Sampling Plan Guidance

increasing probability

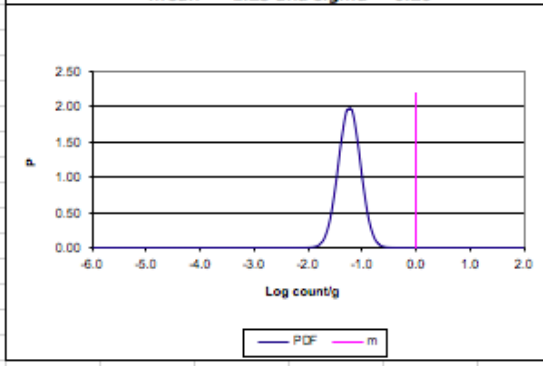
increasing severity

	Conditions expected after sampling		
	Reduction in Cell Density	No Change in Cell Density	Increase in Cell Density
Utility	Case 1	Case 2	Case 3
Indicator	Case 4	Case 5	Case 6
Moderate hazard	Case 7 (n=5, c=2)	Case 8 (n=5, c=1)	Case 9 (n=10, c=1)
Serious hazard	Case 10 (n=5, c=0)	Case 11 (n=10, c=0)	Case 12 (n=20, c=0)
Severe hazard	Case 13 (n=15, c=0)	Case 14 (n=20, c=0)	Case 15 (n=60, c=0)

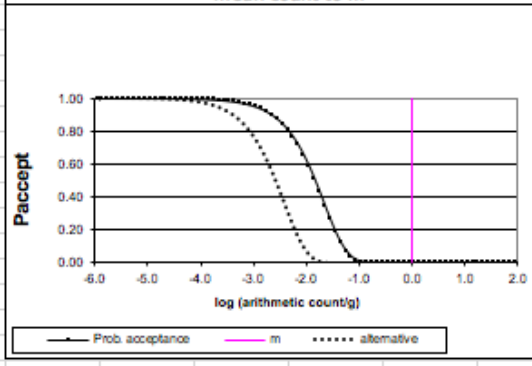
Operating characteristic curve for proportion defective, with n=48 and c=0



Probability density function (PDF) for log counts. Distribution mean = -1.25 and sigma = 0.20



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



Batch acceptance for Pd			P(accept)	
Pd	20	%	0.0	%
actualPd	6.1	%	5.00	%

INPUTS		
mean	-1.25	
sigma	0.20	
m	0.00	
n	48	
c	0	
amount	1	g

P(accept)		
Computed	5.00	%
Desired	5	%

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	-1.25		Computed	0.00 %
sigma	0.20		Target, left	5.00 %
m	-0.72			
n	50			
c	0			
amount	5.2	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	
FSO	-1
complient	0.8921342

Means and median					
	Arithmetic			Geometric=median	
	0.0629	cfu/g		0.0565	cfu/g
one cfu in	15.9	grams	one cfu in	17.7	grams
	-1.20	log cfu/g		-1.25	log cfu/g

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	-0.63
FSO	P exceedance	target level
-0.63	0.0009728	-1.247592

This sampling plan would provide 95 % confidence that a lot of food containing a median concentration of 1 organism in 17.7 g and an average concentration of 1 organism in 15.9 g (and having a standard deviation of 0.20 log cfu/g), would be rejected (i.e. more than 0 out of 48 samples of 1 grams giving detection of the organism)

Use and Interpretation of the ICMSF Sampling Plan Tool

thank you for attention, and questions