FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

Inspiring Trust, Assuring Safe & Nutritious Food

Ministry of Health and Family Welfare, Government of India

Performance of sampling plans for microbiological criteria

Presented by:

Tom Ross

ICMSF

Tasmanian Institute of Agriculture, University of Tasmania

Overview

- Presence / absence testing
- Binomial distribution and ‘simple’ evaluation of a sampling scheme
- The importance of variability
- The ICSMF Sampling Plan Spreadsheet
- Using the Spreadsheet to evaluate sampling plan performance

Presence/Absence Testing

Used when acceptable levels of contamination are very low, and cannot be evaluated by colony plating methods

relies on enrichment of ‘large’ volumes/mass of sample

Presence/Absence Testing

There is no such thing as ‘zero’ contamination -

only some level of confidence that the contamination is below a certain level.

What do we mean by ‘zero tolerance’?

e.g., n= 5, c= 0, m=25g

Binomial Distribution

tells us *how many samples* we need to take (and test) to be *appropriately confident* that the *prevalence of a faulty unit* is below the level we consider to be acceptable

for food microbiology testing, we often define acceptability as the absence of a specific pathogen in a *specified amount* of the food

Probability that no contamination is found

$P_{defective}$	n=1	n=2	n=5
	$1- P_{def} =$	$(1- P_{def})^2=$	$(1- P_{def})^5=$
0.00	1.00	1.00	1.00
0.01	0.99	0.98	0.95
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

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

Binomial Distribution

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:

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

Binomial Distribution

e.g., n= 5, c= 0, m=25g, p = ?

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

$$0.05 = (1 - p)^5$$

$$p = 0.45$$

- *i.e.*, up to 45% of 25 g samples could be contaminated !! or, an 'average' concentration up to 1/56g!
-

But …

in practice, the evaluation of the performance of a microbiological sampling plan is harder than this simple calculation *because*

inhomogenous distribution (log-normal) of organisms among lots of food

simple binomial distribution isn't accurate enough

ICMSF Sampling Plan Spreadsheet

- an ®Excel spreadsheet tool
- can be used to design a sampling plan that detects batches/lots that exceed any specified level of contamination
- variables are:
  - numbers of samples
  - size of samples or sensitivity of detection for each
  - standard deviation of the variability in counts in the lot
  - method sensitivity/specificity

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/publications/software-downloads/

HOME ABOUT US EVENTS IN THE NEWS MEMBERS PUBLICATIONS VIDEO GALLERY CONTACTS LINKS

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)

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

This file can determine the performance of sampling plans for four cases:

- 2 class plans for enrichments (presence/absence, detection of the organism with no count results)	see tab 2class enrichment
- 2 class plans for quantitative determination (like concentration<100 cfu/g)	see tab 2class counts
- 3 class plans for quantitative determination (where concentration is compared to both m and M)	see tab 3class counts
- 2 class plans for enrichments including effects sensitivity and specificity	see tab 2class enrich sensspec
- Effects of sensitivity and specificity (fixed values) are also presented in a tabular form	see tab TableSensSpec

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, hence equals minus log(weight)
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 (only relevant for 2-class enrichement plans)
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). This is usually set at 5%, this then means there is 5% probability of acceptance, or 95% probability of detecting such unacceptable lot
sensitivity	true positive rate= TP/(TP+FN), (assumed to be fixed value depending on the method and not dependant on the concentration of the organisms)
specificity	true negative rate= TN/(TN+FP), (assumed to be fixed value depending on the method and not dependant on the concentration of the organisms)

The Implied Acceptance Level, shown on all 'tabs' (worksheets), allows you to calculate the proportion of all the samples in the lot that would be expected to be above any chosen logCFU value (i.e., a user input). For that logCFU, the z-score is also calculated.

For a manual please open the following word-file

Document

For an explanation on arithmetic and geometric means open the following word-file

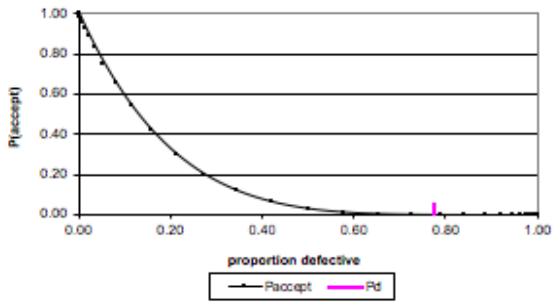
Document

About | Technical issues | Introduction | 2class enrichment | 2class counts | 3class counts | 2class enrich sensspec

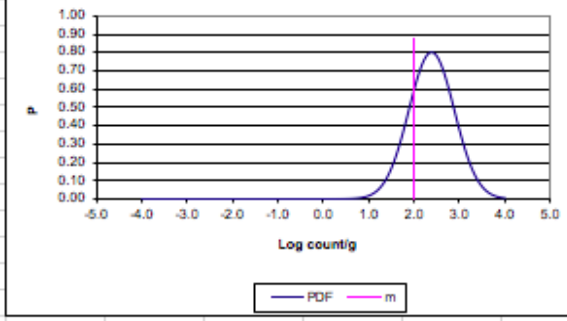
FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

ICMSF Sampling Plan Spreadsheet

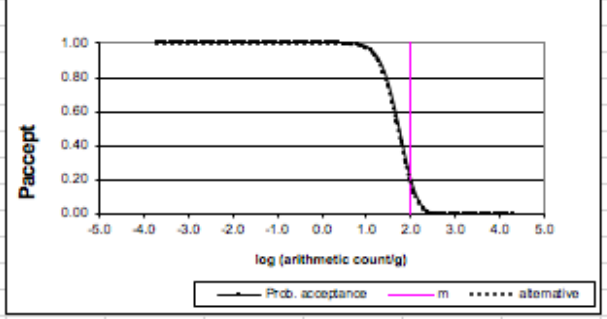
Operating characteristic curve for proportion defective, with n=5 and c=0



Probability density function (PDF) for log counts. Distribution mean = 2.38 and sigma = 0.50



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



Batch acceptance for Pd			P(accept)	
Pd	10	%	59.0	%
actualPd	77.6	%	0.06	%

INPUTS		P(accept)		
mean	2.38	Computed	0.06	%
sigma	0.50	Desired	5	%
m	2			
n	5			
c	0			

Find mean that gives desired P(accept)

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

Project 99.94

ALTERNATIVE n AND c		P(accept)		
mean	2.38	Computed	0.06	%
sigma	0.50	Target, left	0.06	%
m	2.00			
n	5			
c	0			

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	3
complient	0.892505

Means and median			
Arithmetic		Geometric=median	
465.4	cfu/g	239.9	cfu/g
2.67	log cfu/g	2.38	log cfu/g

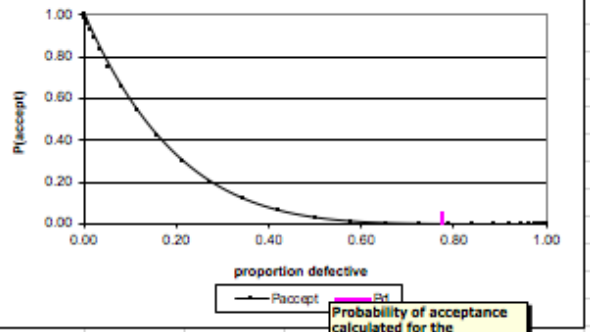
Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	3.93
FSO	P exceedance	target level
3.93	0.0009728	2.3800198

About | Technical issues | Introduction | 2class enrichment | 2class counts | 3class counts | 2class enrich sensspec

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

ICMSF Sampling Plan Spreadsheet (help boxes)

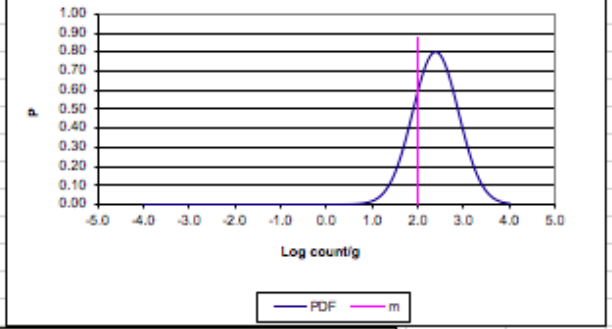
Operating characteristic curve for proportion defective, with n=5 and c=0



Probability of acceptance calculated for the proportion defective entered at left and the sample plan n and c values under INPUTS

Batch acceptance for Pd		
Pd	10	%
actualPd	77.6	%

Probability density function (PDF) for log counts. Distribution mean = 2.38 and sigma = 0.50



Upper boundary of acceptable log(10) count/g. In a 2-class plan there is no marginally acceptable condition. Therefore m = M. All samples giving results exceeding m are defective. Generally a single defective results would cause rejection of the entire lot. (There are no ICMSF 2-class plans where c > 0)

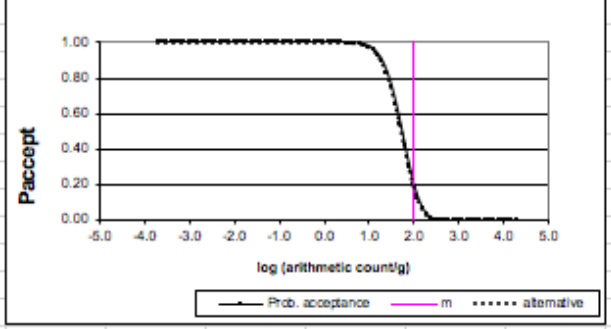
ccept)	0.06	%
	5	%

n that gives P(accept)

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

Project	99.94

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



ALTERNATIVE n AND c		P(accept)		
mean	2.38	Computed	0.06	%
sigma	0.50	Target, left	0.06	%
m	2.00			
n	5			
c	0			

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	3
complient	0.892505

Means and median

Arithmetic		Geometric=median	
465.4	cfu/g	239.9	cfu/g
2.67	log cfu/g	2.38	log cfu/g

Implied Acceptance level

Percentile	z-score	Concentration at this percentile
99.9	3.10	3.93
FSO	P exceedance	target level
3.93	0.0009728	2.3800198

ICMSF Sampling Plan Spreadsheet

- *can also be used to evaluate the detection limits of an existing sampling plan*

Using the ICMSF Sampling Plan Spreadsheet to Assess Sampling Plan Performance

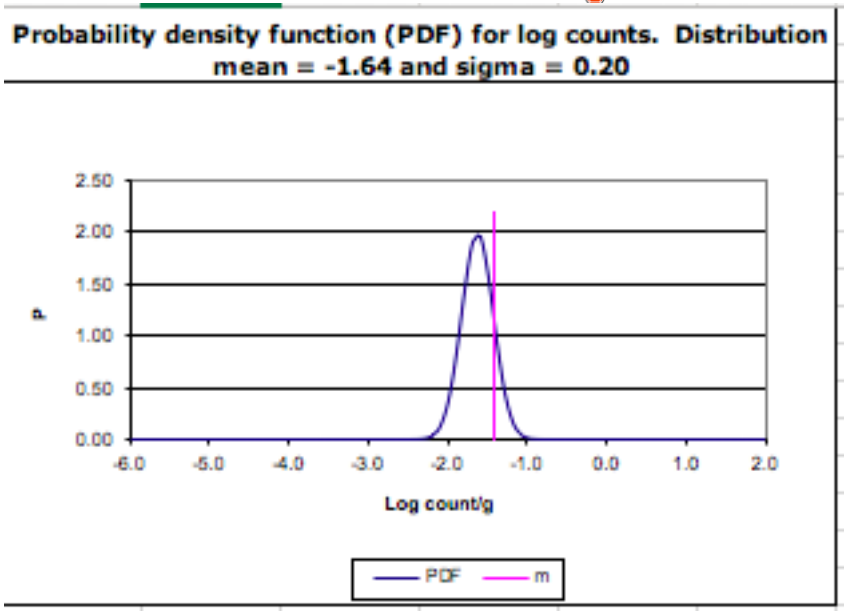
- returning to our earlier example:

- $n = 5$, $c = 0$, $m = 25$ g

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

Using the ICMSF Sampling Plan Spreadsheet to Assess Sampling Plan Performance



INPUTS			P(accept)		
mean	-1.64		Computed	5.00	%

Arithmetic			Geometric=median		
	0.0256	cfu/g		0.0231	cfu/g
one cfu in	39.0	grams	one cfu in	43.3	grams
	-1.59	log cfu/g		-1.64	log cfu/g

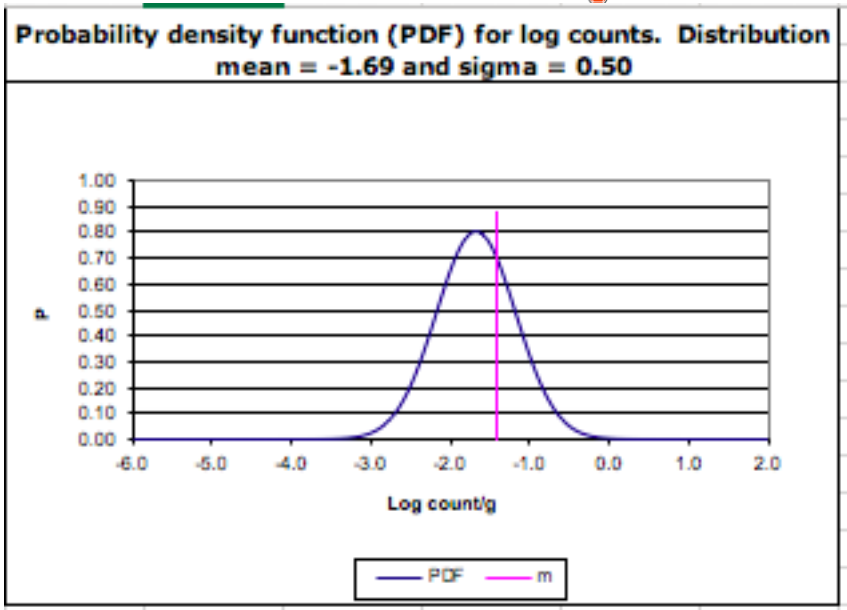
Means and median

Arithmetic			Geometric=median		
	0.0256	cfu/g		0.0231	cfu/g
one cfu in	39.0	grams	one cfu in	43.3	grams
	-1.59	log cfu/g		-1.64	log cfu/g

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

Using the ICMSF Sampling Plan Spreadsheet to Assess Sampling Plan Performance



INPUTS				P(accept)	

	Arithmetic			Geometric=median	
	0.0397	cfu/g		0.0205	cfu/g
one cfu in	25.2	grams	one cfu in	48.9	grams
	-1.40	log cfu/g		-1.69	log cfu/g

Means and median

	Arithmetic			Geometric=median	
	0.0397	cfu/g		0.0205	cfu/g
one cfu in	25.2	grams	one cfu in	48.9	grams
	-1.40	log cfu/g		-1.69	log cfu/g

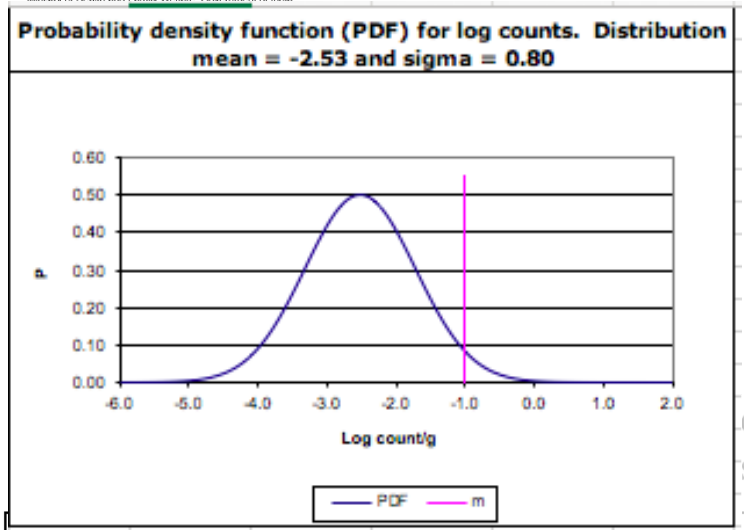
Criteria for pathogenic microorganisms

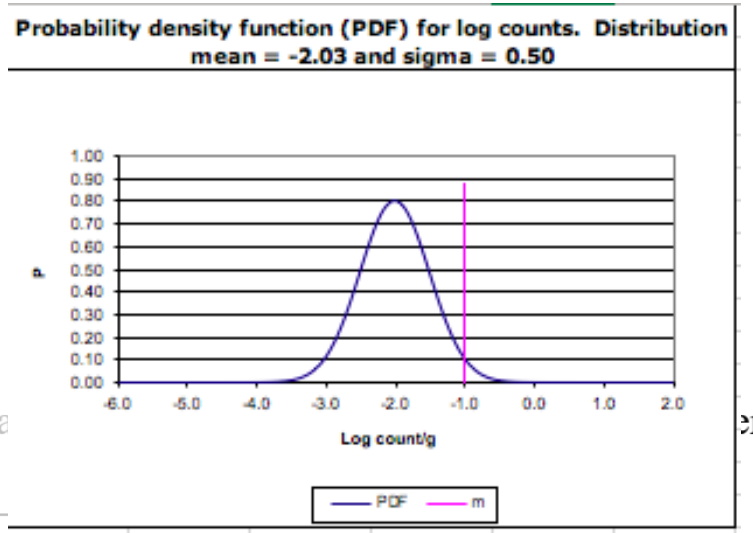
These are to be applied to the finished product (powder form) after primary packaging or anytime thereafter up to the point when the primary package is opened.

Microorganisms	**n**	**c**	**m**	**Class Plan**
Enterobacter sakazakii (Cronobacter species)*	30	0	0/10 g	2
*Salmonella***	60	0	0/25 g	2

Where n = number of samples that must conform to the criteria: c = the maximum allowable number of defective sample units in a 2-class plan. m = a microbiological limit which, in a 2-class plan, separates good quality from defective quality.

*The mean concentration detected is 1 cfu in 340g (if the assumed standard deviation is 0.8 and probability of detection is 95%) or 1 cfu in 100g (if the assumed standard deviation is 0.5 and probability of detection is 99%)





INPUTS		P(accept)		
mean	-2.53	Computed	5.00	%
sigma	0.80	Desired	5	%
m	-1.00	Find mean that gives desired P(accept)		
n	30			
c	0	Find n that gives		

INPUTS		P(accept)		
mean	-2.03	Computed	1.00	%
sigma	0.50	Desired	1	%
m	-1.00	Find mean that gives desired P(accept)		
n	30			
c	0	Find n that gives		

Means and median					
	Arithmetic			Geometric=median	
	0.0160	cfu/g		0.0029	cfu/g
one cfu in	62.6	grams	one cfu in	341.5	grams
	-1.80	log cfu/g		-2.53	log cfu/g

Means and median				
Arithmetic			Geometric=median	
0.0181	cfu/g		0.0094	cfu/g
55.1	grams	one cfu in	106.9	grams
-1.74	log cfu/g		-2.03	log cfu/g

*The mean concentration detected is 1 cfu in 340g (if the assumed standard deviation is 0.8 and probability of detection is 95%) or 1 cfu in 100g (if the assumed standard deviation is 0.5 and probability of detection is 99%)

Criteria for pathogenic microorganisms

These are to be applied to the finished product (powder form) after primary packaging or anytime thereafter up to the point when the primary package is opened.

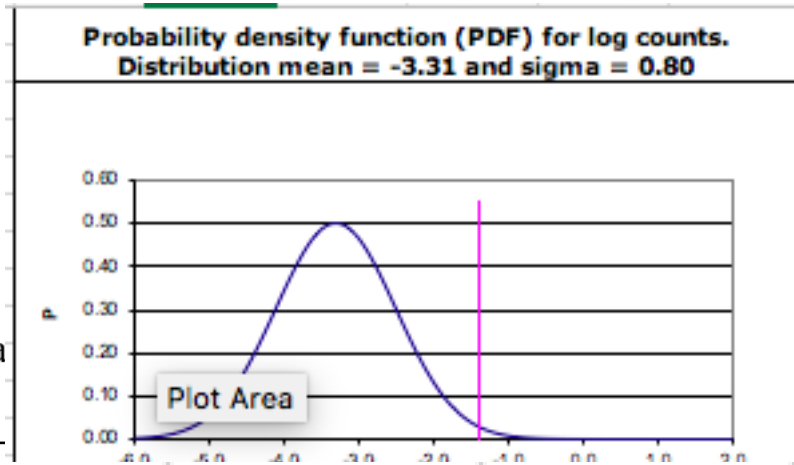
Microorganisms	**n**	**c**	**m**	**Class Plan**
Enterobacter sakazakii (Cronobacter species)*	30	0	0/10 g	2
*Salmonella***	60	0	0/25 g	2

Where n = number of samples that must conform to the criteria: c = the maximum allowable number of defective sample units in a 2-class plan. m = a microbiological limit which, in a 2-class plan, separates good quality from defective quality.

*The mean concentration detected is 1 cfu in 340g (if the assumed standard deviation is 0.8 and probability of detection is 95%) or 1 cfu in 100g (if the assumed standard deviation is 0.5 and probability of detection is 99%)

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



Criteria for pathogenic microorganisms

These are to be applied to the finished product (powder form) a
up to the point when the primary package is opened.

Means and median					
	Arithmetic			Geometric=median	
	0.0027	cfu/g		0.0005	cfu/g
one cfu in	372.9	grams	one cfu in	2034.2	grams
	-2.57	log cfu/g		-3.31	log cfu/g

defective sample units in a 2-class plan. m = a microbiological
quality from defective quality.

*The mean concentration detected is 1 cfu in 340g (if the assur
of detection is 95%) or 1 cfu in 100g (if the assumed standard d
99%)

amount	25	g		Find n that gives desired P(accept) or better (less)	
				Project	95.00

Means and median					
	Arithmetic			Geometric=median	
	0.0027	cfu/g		0.0005	cfu/g
one cfu in	372.9	grams	one cfu in	2034.2	grams
	-2.57	log cfu/g		-3.31	log cfu/g

FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA

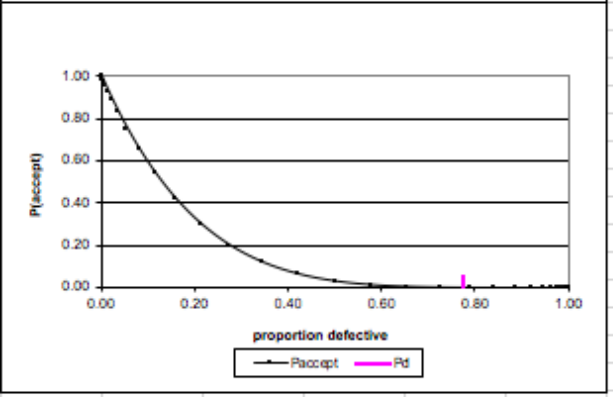
Inspiring Trust, Assuring Safe & Nutritious Food

Ministry of Health and Family Welfare, Government of India

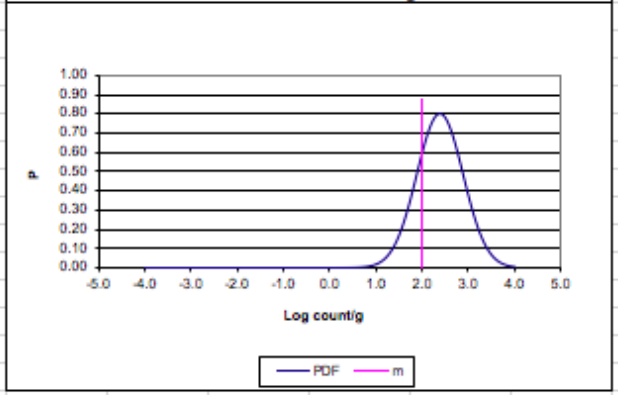
CHIFSS

CII-HUL Initiative on Food Safety Sciences

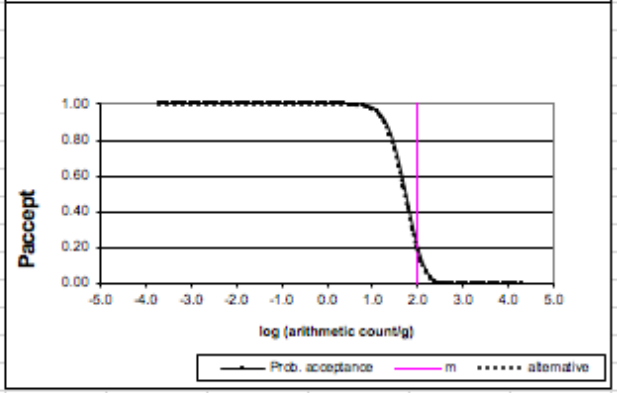
Operating characteristic curve for proportion defective, with n=5 and c=0



Probability density function (PDF) for log counts. Distribution mean = 2.38 and sigma = 0.50



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



Batch acceptance for Pd			P(accept)	
Pd	10	%	59.0	%
actualPd	77.6	%	0.06	%

INPUTS	
mean	2.38
sigma	0.50
m	2
n	5
c	0

P(accept)		
Computed	0.06	%
Desired	5	%

Find mean that gives desired P(accept)

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

Project 99.94

ALTERNATIVE n AND c	
mean	2.38
sigma	0.50
m	2.00
n	5
c	0

P(accept)		
Computed	0.06	%
Target, left	0.06	%

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	3
compliant	0.892505

Means and median			
Arithmetic		Geometric=median	
465.4	cfu/g	239.9	cfu/g
2.67	log cfu/g	2.38	log cfu/g

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	3.93
FSO	P exceedance	target level
3.93	0.0009728	2.3800198

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

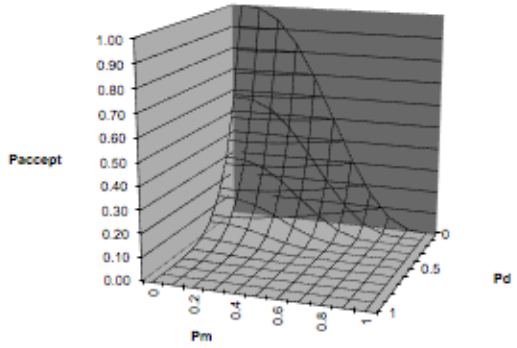
Criteria for hygiene indicators in milk powder processing (3 class enumeration)

Microorganisms	n	c	m	M	Class Plan
Mesophilic Aerobic Bacteria*	5	2	500/g	5000/g	3

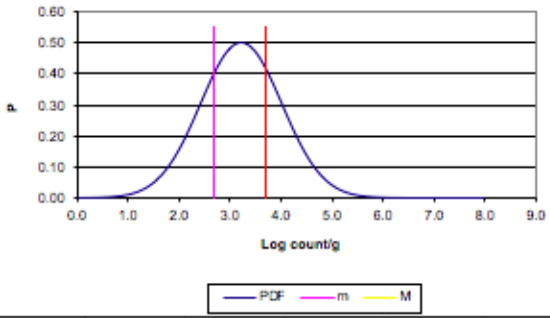
FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA
Inspiring Trust, Assuring Safe & Nutritious Food
Ministry of Health and Family Welfare, Government of India

CHIFSS
CII-HUL Initiative on Food Safety Sciences

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 = 3.22 and sigma = 0.80



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

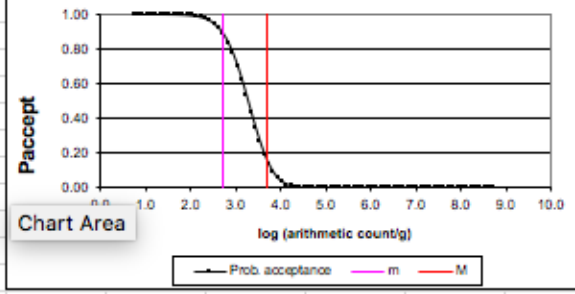


Chart Area

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

INPUTS	
mean	3.22
sigma	0.8
m	2.69897
M	3.69897
n	5
c	2

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	
mean	3.22
sigma	0.80
m	2.70
M	3.70
n	10
c	0

P(accept)		
Computed	0.00	%
Target, left	5.00	%

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	5
compliant	0.9871627

Means and median			
Arithmetic		Geometric=median	
8952.6	cfu/g	1641.1	cfu/g
3.95	log cfu/g	3.22	log cfu/g

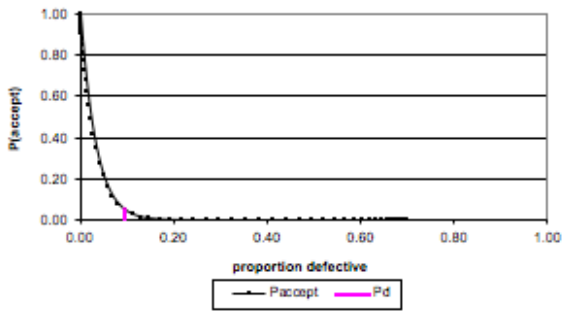
Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	5.69
FSO	P exceedance	target level
5.69	0.0009728	3.2151223

This sampling plan would provide 95 % confidence that a lot of food containing a median concentration of 1,641.1 cfu/g and an average concentration of 8,952.6 cfu/g (having a a standard deviation of 0.80 log cfu/g), would be rejected (i.e. either more than 2 out of 5 samples having higher levels than 2.7 logs cfu/g or 1 or more samples higher than 3.7 log cfu/g)

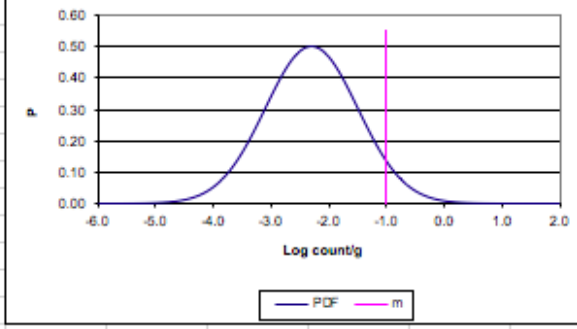
* mesophiles: 3 class counts:1641 cfu/g (8952 cfu/g arithmetic)

Can also consider method sensitivity and specificity …

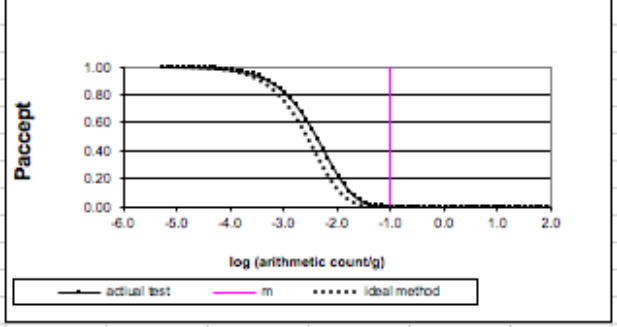
Operating characteristic curve for proportion defective, with n=30 and c=0



Probability density function (PDF) for log counts. Distribution mean = -2.31 and sigma = 0.80



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



Batch acceptance for Pd			P(accept)	
Pd	20	%	0.1	%
actualPd	9.5	%	5.00	%

INPUTS		
mean	-2.31	
sigma	0.80	
m	-1.00	
n	30	
c	0	
amount	10	g
sensitivity	0.7	
specificity	1	

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

Ideal method	1.26 %
Actual test	5.00 %

specificity and sensitivity of the test are assumed to be fixed values and not dependant on the concentration of the organisms.

Sandbox: for your own calculations

FSO	-1
complient	0.9495054

Means and median

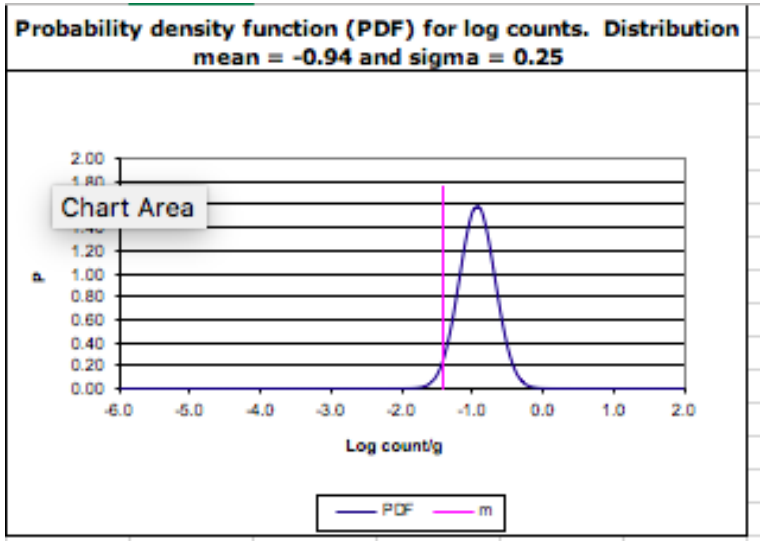
	Arithmetic			Geometric=median	
	0.0266	cfu/g		0.0049	cfu/g
one cfu in	37.6	grams	one cfu in	205.1	grams
	-1.58	log cfu/g		-2.31	log cfu/g

Implied Acceptance level

Percentile	z-score	Concentration at this percentile
99.9	3.10	0.17
FSO	P exceedance	target level
0.17	0.0009728	-2.312062

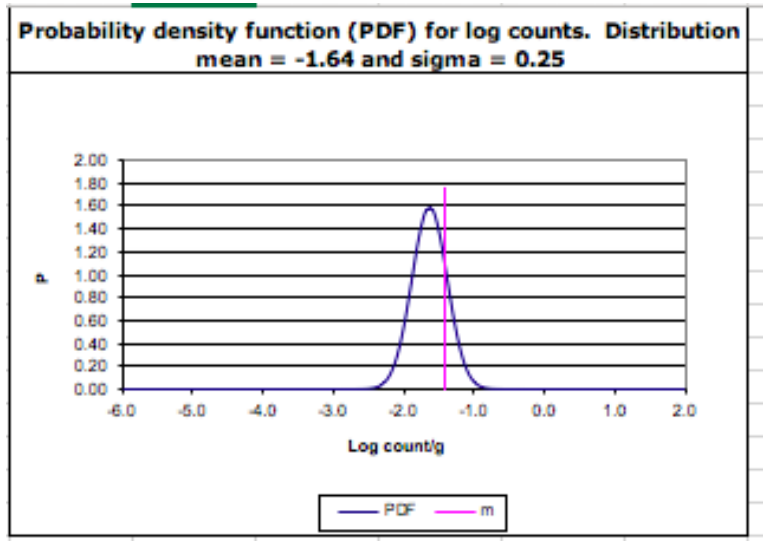
This sampling plan would provide 95 % confidence that a lot of food containing a median concentration of 1 organism in 205.1 g and an average concentration of 1 organism in 37.6 g (and having a standard deviation of 0.80 log cfu/g), would be rejected (i.e. more than 0 out of 30 samples of 10 grams giving detection of the organism) for method with sensitivity=0.7 and selectivity=1

Can also consider method sensitivity and specificity …



INPUTS			P(accept)		
mean	-0.94		Computed	5.00	%
sigma	0.25		Desired	5	%
m	-1.40		Find mean that gives desired P(accept)		
n	5				
c	0				
amount	25	g	Find n that gives desired P(accept) or better (less)		
sensitivity	0.5				
specificity	1		Preject	95.00	

Means and median					
	Arithmetic			Geometric=median	
	0.1341	cfu/g		0.1136	cfu/g
one cfu in	7.5	grams	one cfu in	8.8	grams
	-0.87	log cfu/g		-0.94	log cfu/g



INPUTS			P(accept)		
mean	-1.64		Computed	5.00	%
sigma	0.25		Desired	5	%
m	-1.40		Find mean that gives desired P(accept)		
n	5				
c	0				
amount	25	g	Find n that gives desired P(accept) or better (less)		
sensitivity	1				
specificity	1		Preject	95.00	

Means and median					
	Arithmetic			Geometric=median	
	0.0267	cfu/g		0.0227	cfu/g
one cfu in	37.4	grams	one cfu in	44.1	grams
	-1.57	log cfu/g		-1.64	log cfu/g

Conclusions

- understanding the probability of detecting an organism in a sample can be used to quantify the detection limits of sampling schemes
- the ICSMF Sampling Plan spreadsheet automates this process
- may need to make some assumptions about SD in the lot, and reliability of the method

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

Thank you for your attention …