

# Using the ICMSF Sampling Plan Tool to assess the performance of a Microbiological Criteria

## **Part 2: MC for *Listeria monocytogenes* in RTE Foods not supporting growth**



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*ICMSF Member 2001-present*

# Outline

- The Microbiological Criterion (MC) for Ready-to-Eat (RTE) foods that do not support growth of *Listeria monocytogenes* (*Lm*)
- Calculate the performance of the sampling plan with the ICMSF sampling plan tool
- Using the tool for further interpretations of the MC
  - Changing the standard deviation
  - Changing the confidence level

# MC: RTE foods not supporting Lm growth\*

## Microbiological criterion for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

Point of application	Microorganism	<i>n</i>	<i>c</i>	<i>m</i>	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	<i>Listeria monocytogenes</i>	5 <sup>a</sup>	0	100 cfu/g <sup>b</sup>	2 <sup>c</sup>

Where *n* = number of samples that must conform to the criterion; *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 acceptable lots from unacceptable lots.

<sup>a</sup> National governments should provide or support the provision of guidance on how samples should be collected and handled, and the degree to which compositing of samples can be employed.

<sup>b</sup> This criterion is based on the use of the ISO 11290-2 method.

Other methods that provide equivalent sensitivity, reproducibility, and reliability can be employed if they have been appropriately validated (e.g., based on ISO 16140).

<sup>c</sup> Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 93.3 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected based on any of the five samples exceeding 100 cfu/g *L. monocytogenes*.

Such a lot may consist of 55% of the samples being below 100 cfu/g and up to 45% of the samples being above 100 cfu/g, whereas 0.002% of all the samples from this lot could be above 1000 cfu/g.

The typical actions to be taken where there is a failure to meet the above criterion would be to (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, and/or (3) determine and correct the root cause of the failure.

\*CAC/GL61-2007, "Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods"

# MC: RTE foods not supporting Lm growth

## Microbiological criterion for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

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Such a lot may consist of 55% of the samples being below 100 cfu/g and up to 45% of the samples being above 100 cfu/g, whereas 0.002% of all the samples from this lot could be above 1000 cfu/g.

# Where to find the tool?



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# The ICMSF sampling plan tool



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**Microbiological sampling plans: a tool to explore ICMSF recommendations**

Version 2.10: Copyright 1998 David Legan & Mark Vandeven 2009 Marcel Zwietering & Peter S

**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	Version 2.01: Addition of enrichment calculations	Version 2.02:
Mark Vandeven and David Legan	Marcel Zwietering	This version sim
Nabisco Research	Wageningen University	with Marcel Zwi
R M Schaeberle Technology Center	Laboratory of Food Microbiology	
200 DeForest Avenue	PO Box 17	<b>Version 2.03-2</b>
East Hanover, NJ 07936-1944, USA	6700AA Wageningen	The file is furth
email: VandevenM@Nabisco.com, or	The Netherlands	
LeganD@Nabisco.com	email: Marcel.Zwietering@WUR.nl	<b>Version 2.07: C</b>
		<b>Version 2.08: S</b>
		<b>Version 2.09: S</b>
		<b>Version 2.10: S</b>

**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)

Microbial testing in food safety: effect of specificity and sensitivity on sampling plans — how does the OC curve move

M.H. Zwietering, H.M.W. den Besten.

Current Opinion Food Science 12, 42–51 (2016)

**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 09-05-20

update 2009-10-7: reduced further in calculations, removed empty modules, updated MZ

update 2009-10-12: returned to goalseek, no need for solver and analytic toolpack

update 2009-10-14: limited c maximally at 1e10 cfu/g in poissonlognormal

update 2009-10-15: limited sigma >0.05

update 2009-10-27: protected help file in Introduction

update 2010-10-19: added log arithmetic means

update 2013-11-25: version 07: added boxes with performance, changed graph to arithmetic mean

update 2014-10-23: version 08: sensitivity and selectivity added and boxes placed

**2-class counts**

Technical issues Introduction 2-class enrich counts 3-class mixed 2-class enrich sensspec TableSensSpec

**DISCLAIMER**

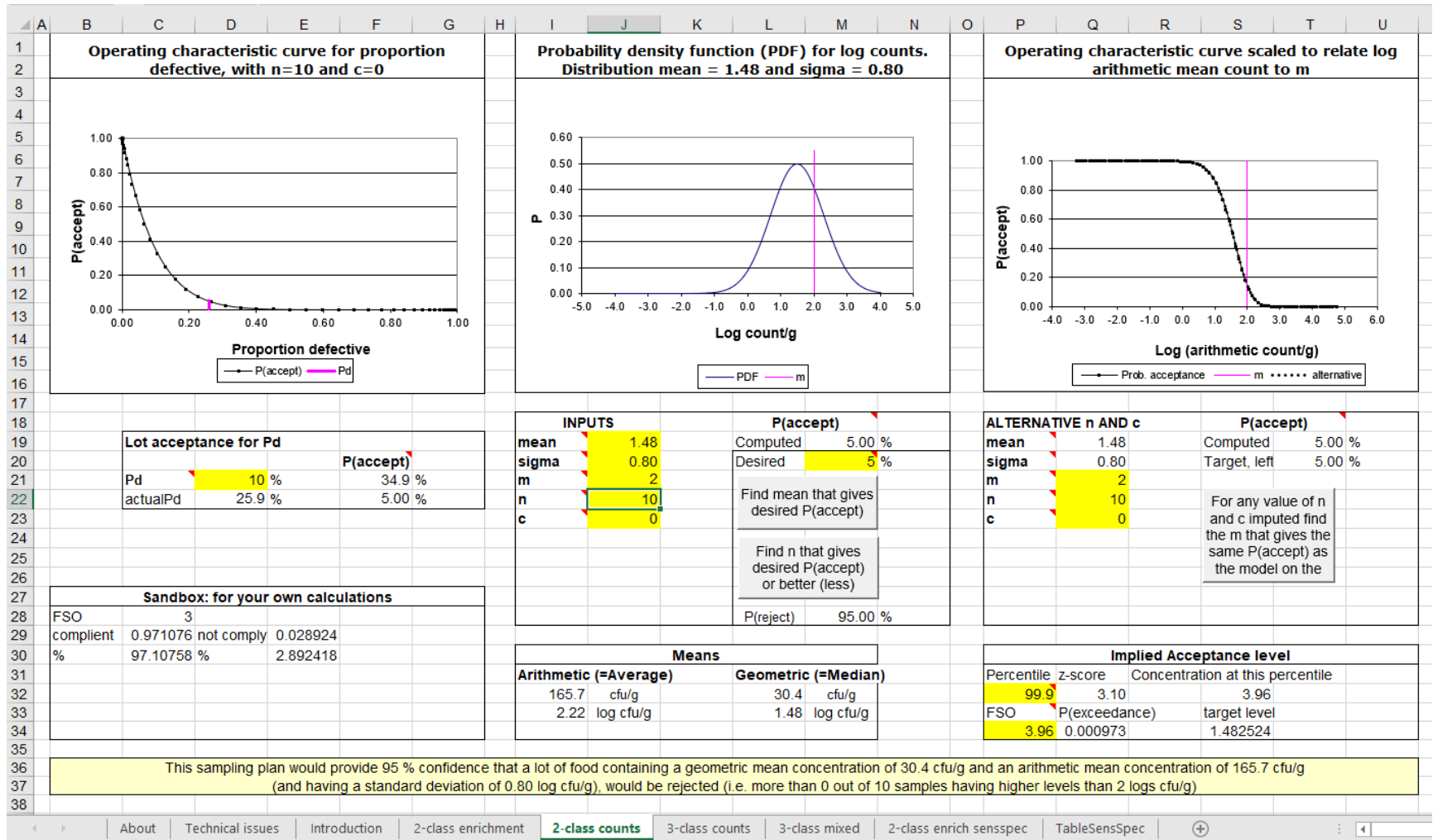
The authors have taken every care to ensure that the output from this workbook is accurate. In making this tool available to promote discussion and understanding of the power and limitations of attributes sampling plans neither the authors nor ICMSF accept any liability for any consequences, direct or indirect resulting from a decision by the user to take, or not to take, any action based on an output from this workbook.

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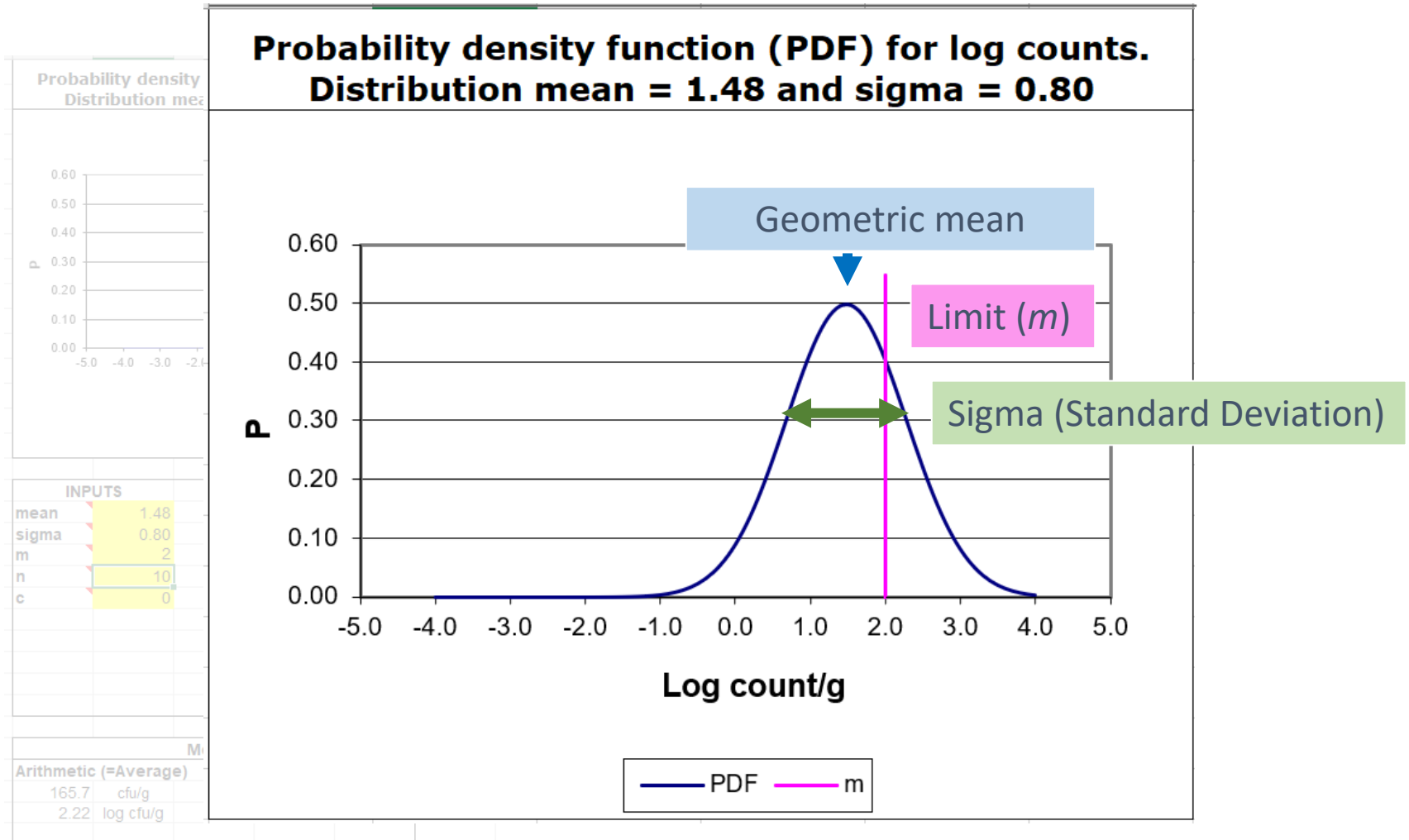
[About](#) Technical issues Introduction 2-class enrich counts 3-class mixed 2-class enrich sensspec TableSensSpec



# The 2-class counts dashboard

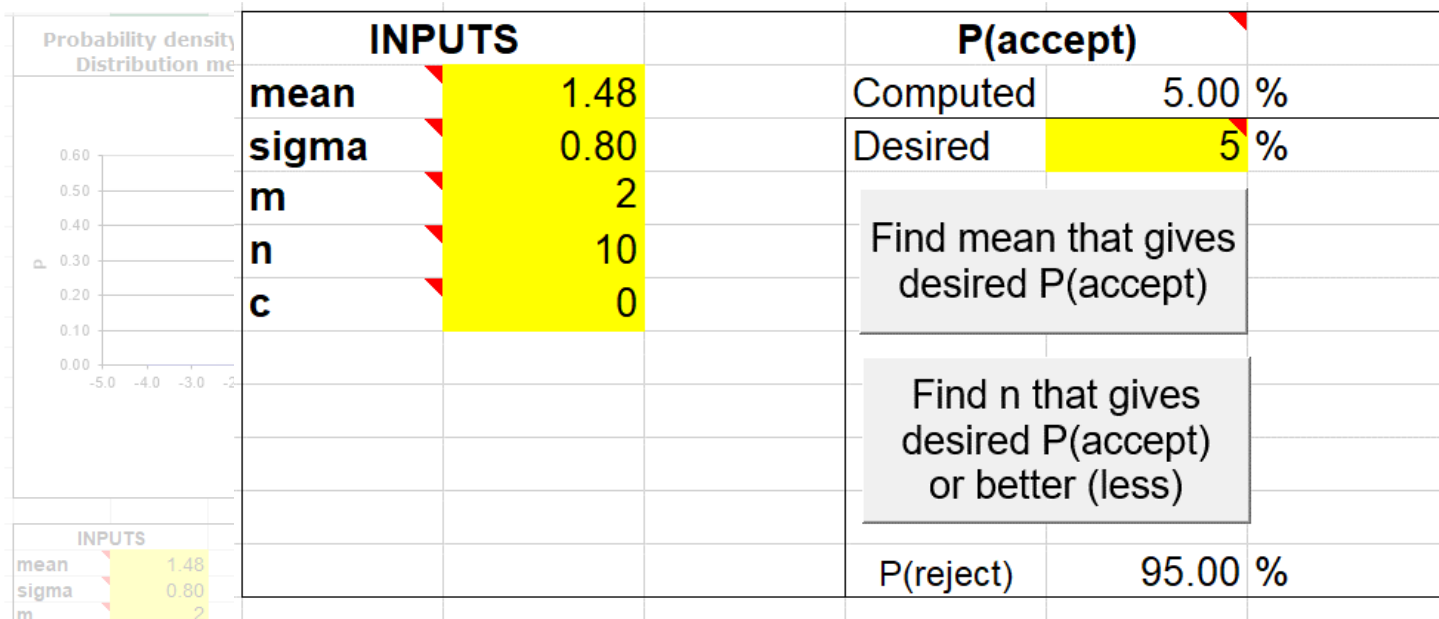


# The 2-class counts dashboard

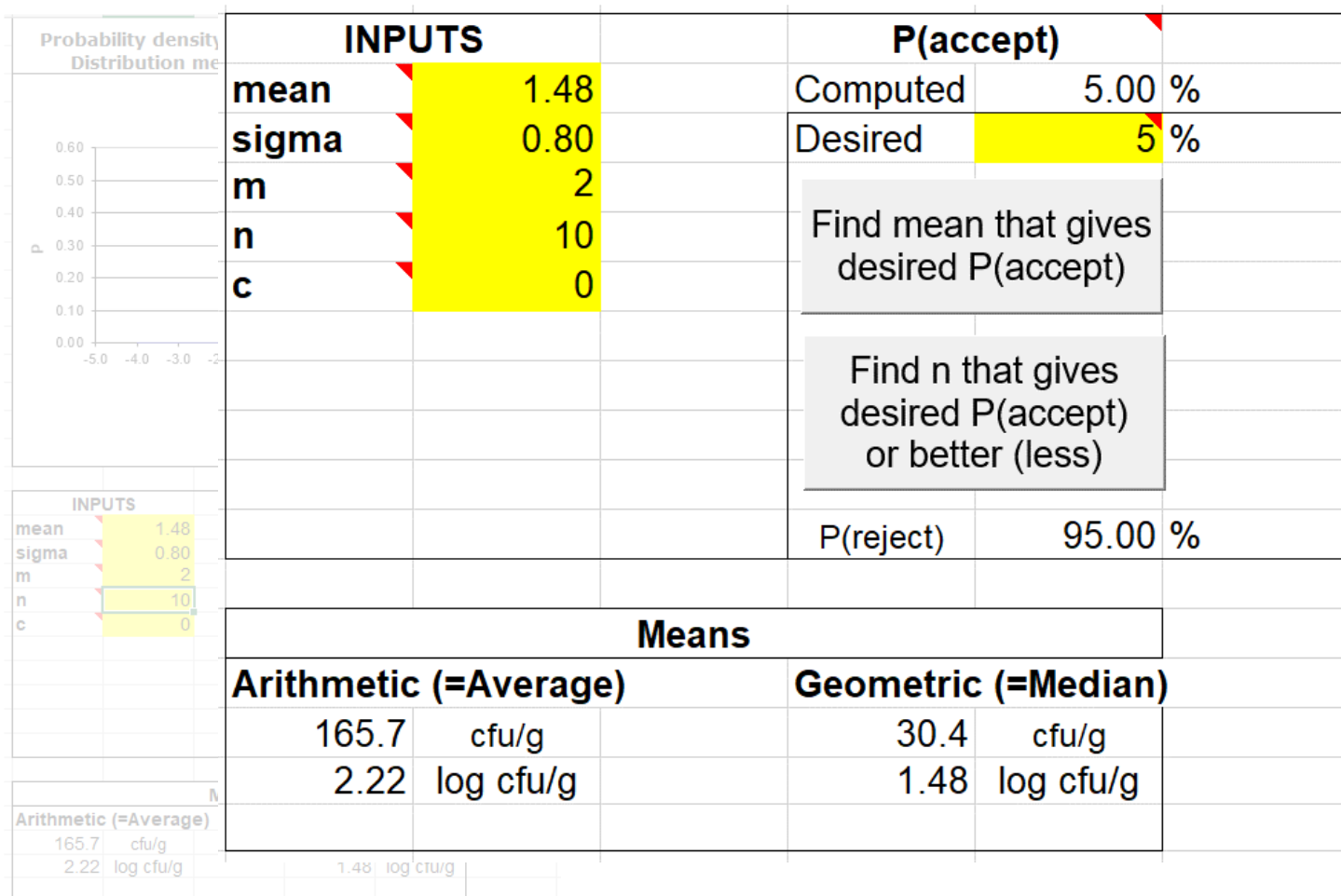




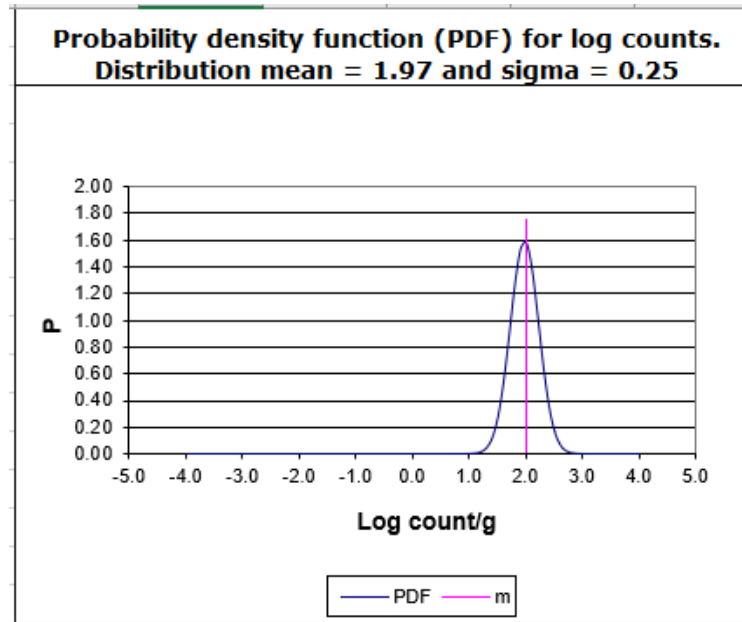
# The 2-class counts dashboard



# The 2-class counts dashboard



# The Lm MC in the 2-class counts dashboard



Calculated mean and distribution

Standard deviation

$n, c, m$  from sampling plan

INPUTS		P(accept)	
mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %
m	2	Find mean that gives desired P(accept)	
n	5	Find n that gives desired P(accept) or better (less)	
c	0	P(reject)	95.00 %

Chance to accept a non-compliant lot

Chance to reject a non-compliant lot

Means			
Arithmetic (=Average)		Geometric (=Median)	
109.9	cfu/g	93.1	cfu/g
2.04	log cfu/g	1.97	log cfu/g

# The Lm MC in the 2-class counts dashboard

INPUTS		P(accept)	
mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %
m	2	Find mean that gives desired P(accept)	
n	5	Find n that gives desired P(accept) or better (less)	
c	0	P(reject)	95.00 %
<b>Means</b>			
<b>Arithmetic (=Average)</b>		<b>Geometric (=Median)</b>	
109.9	cfu/g	93.1	cfu/g
2.04	log cfu/g	1.97	log cfu/g

This sampling plan would provide 95 % confidence that a lot of food containing a geometric mean concentration of 93.1 cfu/g and an arithmetic mean concentration of 109.9 cfu/g (and having a standard deviation of 0.25 log cfu/g), would be rejected (i.e. more than 0 out of 5 samples having higher levels than 2 logs cfu/g)

This sampling plan would provide

- 95% confidence that a lot of food containing a
- geometric mean concentration of 93.1 cfu/g and an
- arithmetic mean concentration of 109.9 cfu/g
- (and having a standard deviation of 0.25 log cfu/g),
- would be rejected
- (i.e. more than 0 out of 5 samples having higher levels than 2 logs cfu/g)

# The Lm MC in the 2-class counts dashboard

INPUTS		P(accept)	
mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %
m	2	Find mean that gives desired P(accept)	
n	5	Find n that gives desired P(accept) or better (less)	
c	0	P(reject)	95.00 %
<b>Means</b>			
<b>Arithmetic (=Average)</b>		<b>Geometric (=Median)</b>	
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# MC: RTE foods not supporting Lm growth

## Microbiological criterion for ready-to-eat foods in which growth of *L. monocytogenes* will not occur

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# MC: RTE foods not supporting Lm growth

INPUTS		P(accept)		ALTERNATIVE n AND c		P(accept)	
mean	1.97	Computed	5.00 %	mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %	sigma	0.25	Target, left	5.00 %
m	2	Find mean that gives desired P(accept)		m	2	For any value of n and c imputed find the m that gives the same P(accept) as the model on the	
n	5			n	5		
c	0	Find n that gives desired P(accept) or better (less)		c	0		
				P(reject)		95.00 %	

Means	
<b>Arithmetic (=Average)</b>	<b>Geometric (=Median)</b>
109.9 cfu/g	93.1 cfu/g
2.04 log cfu/g	1.97 log cfu/g

Implied Acceptance level		
Percentile	z-score	Concentration at this percentile
99.9	3.10	2.74
FSO	P(exceedance)	target level
2.00	0.450717	1.225399

**level for which the probability of exceedance is calculated**

FSO	P(exceedance)
2.00	0.450717

Enter concentration in log cfu/g

# MC: RTE foods not supporting Lm growth

INPUTS		P(accept)		ALTERNATIVE n AND c		P(accept)	
mean	1.97	Computed	5.00 %	mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %	sigma	0.25	Target, left	5.00 %
m	2	Find mean that gives desired P(accept)		m	2	For any value of n and c imputed find the m that gives the same P(accept) as the model on the	
n	5			n	5		
c	0	Find n that gives desired P(accept) or better (less)		c	0		
				P(reject)		95.00 %	

Means	
Arithmetic (=Average)	Geometr
109.9 cfu/g	93.
2.04 log cfu/g	1.9

$f_x$

$$=1-NORM.DIST(P34,meanlog,sigma,1)$$

Tool calculates P(exceedance)

FSO	P(exceedance)
2.00	0.450717



# MC: RTE foods not supporting Lm growth

Concentration  
(cfu/g)

P(exceed)  
calculation

Proportion of food lot over  
chosen concentration

10	<table border="1"> <thead> <tr> <th>FSO</th> <th>P(exceedance)</th> </tr> </thead> <tbody> <tr> <td>1.00</td> <td>0.999947</td> </tr> </tbody> </table>	FSO	P(exceedance)	1.00	0.999947	99.99 %
FSO	P(exceedance)					
1.00	0.999947					
100	<table border="1"> <thead> <tr> <th>FSO</th> <th>P(exceedance)</th> </tr> </thead> <tbody> <tr> <td>2.00</td> <td>0.450717</td> </tr> </tbody> </table>	FSO	P(exceedance)	2.00	0.450717	45.07 %
FSO	P(exceedance)					
2.00	0.450717					
500	<table border="1"> <thead> <tr> <th>FSO</th> <th>P(exceedance)</th> </tr> </thead> <tbody> <tr> <td>2.70</td> <td>0.001729</td> </tr> </tbody> </table>	FSO	P(exceedance)	2.70	0.001729	0.17 %
FSO	P(exceedance)					
2.70	0.001729					
1000	<table border="1"> <thead> <tr> <th>FSO</th> <th>P(exceedance)</th> </tr> </thead> <tbody> <tr> <td>3.00</td> <td>1.86E-05</td> </tr> </tbody> </table>	FSO	P(exceedance)	3.00	1.86E-05	0.00186 %
FSO	P(exceedance)					
3.00	1.86E-05					
5000	<table border="1"> <thead> <tr> <th>FSO</th> <th>P(exceedance)</th> </tr> </thead> <tbody> <tr> <td>3.70</td> <td>2.2E-12</td> </tr> </tbody> </table>	FSO	P(exceedance)	3.70	2.2E-12	0.00000000022 %
FSO	P(exceedance)					
3.70	2.2E-12					

# MC performance: Changing assumptions

**Microbiological criterion for ready-to-eat foods  
in which growth of *L. monocytogenes* will not occur**


$n$	$c$	$m$	Class Plan
$5^a$	0	100 cfu/g <sup>b</sup>	$2^c$

Standard Deviation (SD) = sigma = 0.25 log cfu/g

Confidence Level (CL) = P(reject) = 95%

# Changing assumptions: standard deviation (SD)

<b>Sigma</b>									
0.25									
0.50									
0.80									
1.20									
1.50									



INPUTS				P(accept)	
mean		1.97		Computed	5.00 %
sigma		0.25		Desired	5 %
m		2		Find mean that gives desired P(accept)	
n		5			
c		0			
				Find n that gives desired P(accept) or better (less)	
				P(reject)	95.00 %
<b>Means</b>					
<b>Arithmetic (=Average)</b>			<b>Geometric (=Median)</b>		
109.9	cfu/g		93.1	cfu/g	
2.04	log cfu/g		1.97	log cfu/g	

Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Changing assumptions: standard deviation (SD)

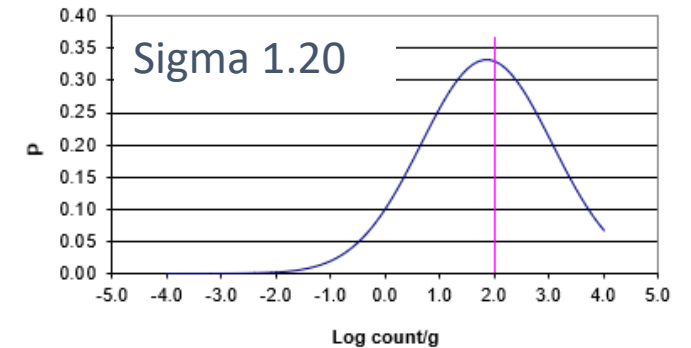
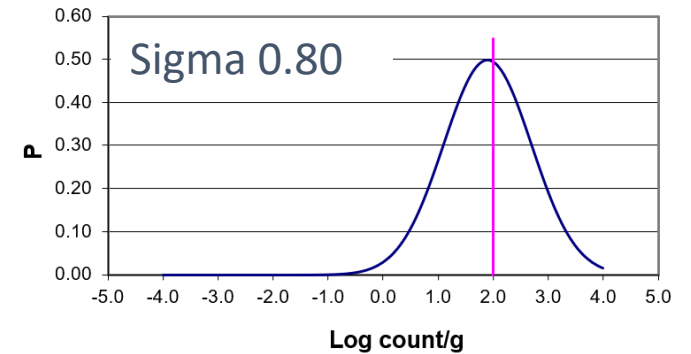
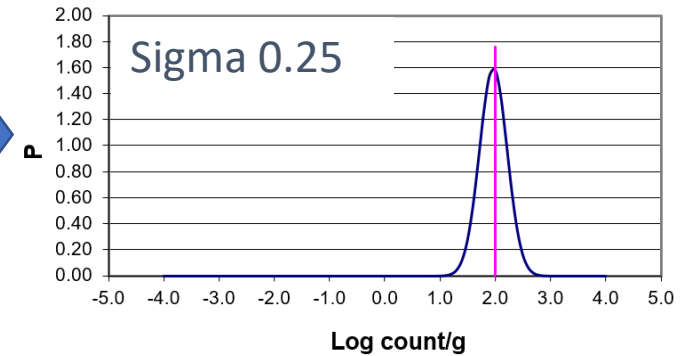
<b>Sigma</b>	<b>0.25</b>	<b>0.50</b>	<b>0.80</b>	<b>1.20</b>	<b>1.50</b>
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INPUTS		P(accept)	
mean	1.97	Computed	5.00 %
sigma	0.25	Desired	5 %
m	2	Find mean that gives desired P(accept)	
n	5		
c	0		
Find n that gives desired P(accept) or better (less)			
		P(reject)	95.00 %

Means			
Arithmetic (=Average)		Geometric (=Median)	
109.9	cfu/g	93.1	cfu/g
2.04	log cfu/g	1.97	log cfu/g



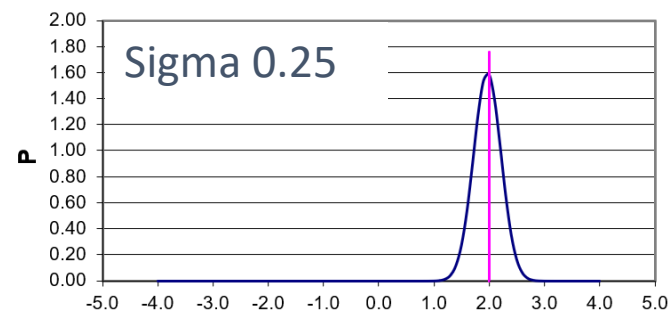
Confidence Level (CL) is 95%  
 Sigma represents Standard Deviation (SD)

# Changing assumptions: standard deviation (SD)

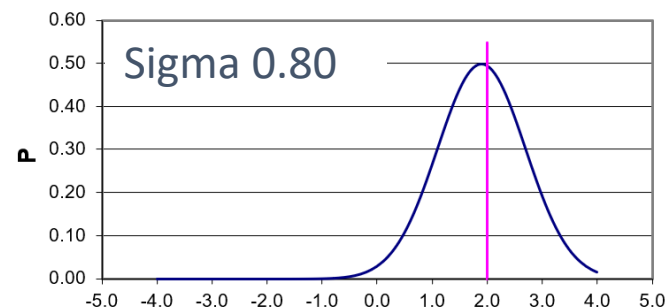


Sigma	Arithmetic mean		Geometric mean	
	<i>log cfu/g</i>	<i>cfu/g</i>	<i>log cfu/g</i>	<i>cfu/g</i>
<b>0.25</b>			<b>93.1</b>	<b>1.97</b>
<b>0.50</b>			<b>86.7</b>	<b>1.94</b>
<b>0.80</b>			<b>79.6</b>	<b>1.90</b>
<b>1.20</b>			<b>71.0</b>	<b>1.85</b>

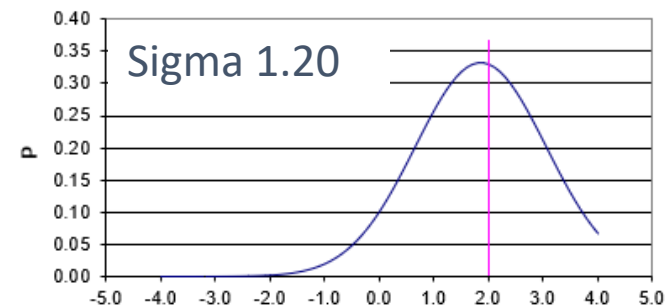
Limit  $m = 2 \log \text{cfu/g}$



Log count/g



Log count/g



Log count/g

Confidence Level (CL) is 95%

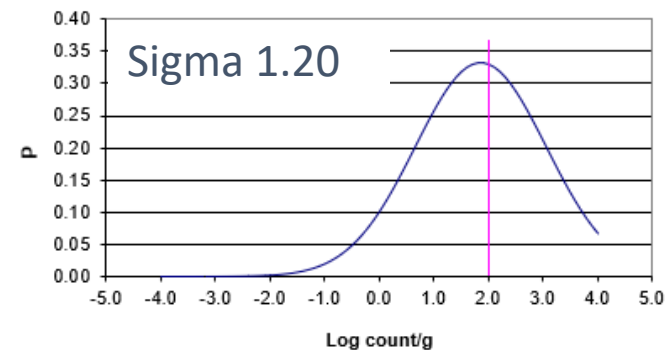
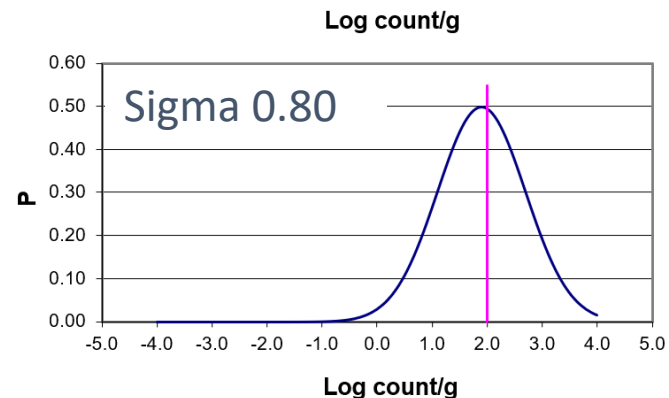
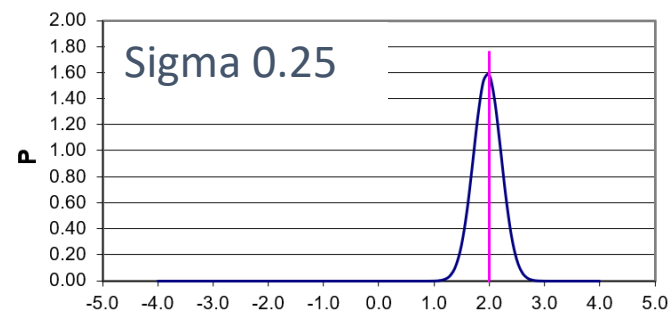
Sigma represents Standard Deviation (SD)

# Changing assumptions: standard deviation (SD)

Codex

Sigma	Arithmetic mean		Geometric mean	
	<i>log cfu/g</i>	<i>cfu/g</i>	<i>log cfu/g</i>	<i>cfu/g</i>
<b>0.25</b>	110	2.04	93.1	1.97
<b>0.50</b>	168	2.23	86.7	1.94
<b>0.80</b>	434	2.64	79.6	1.90
<b>1.20</b>	3230	3.51	71.0	1.85

Limit  $m = 2 \log \text{cfu/g}$



Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Impact of sigma on lot status

Calculate using P(exceedance)

Sigma (log cfu/g)	Estimated Lm levels in proportions (%) of a food lot				
	≤ 100 (2 log)	> 100 (2 log)	> 1000 (3 log)	> 10000 (4log)	> 100000 (5 log)
<b>0.25</b>	55 %	45 %	0.002 %	0.00000	0.00000
<b>0.80</b>	55 %	45 %	8.5 %	0.43 %	0.00536 %
<b>1.20</b>	55 %	45 %	16.9 %	3.67 %	0.435 %



Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Changing assumptions: confidence level

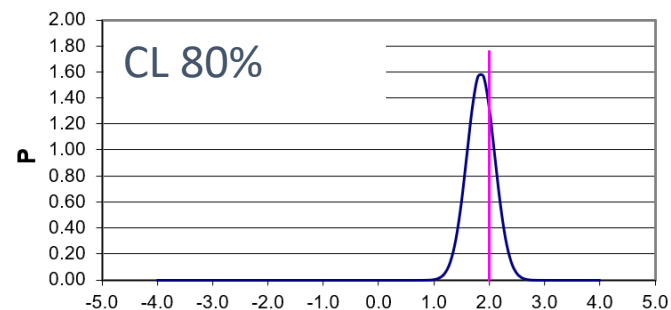
Confidence Level (%)	Arithmetic mean		Geometric mean	
	<i>cfu/g</i>	<i>log cfu/g</i>	<i>cfu/g</i>	<i>log cfu/g</i>
80.00			70.90	1.85
90.00			82.50	1.92
<b>95.00</b>			93.10	1.97
99.00			116.00	2.06
99.90			147.00	2.17



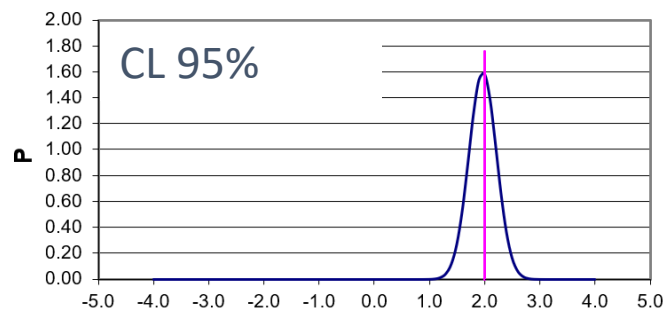
Limit  $m = 2 \log \text{cfu/g}$

Standard Deviation is 0.25 log cfu/g

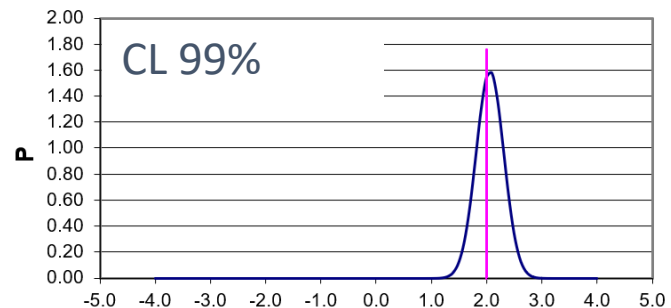
Confidence Level (CL) represents P(reject)



Log count/g



Log count/g



Log count/g



# Changing assumptions: confidence level

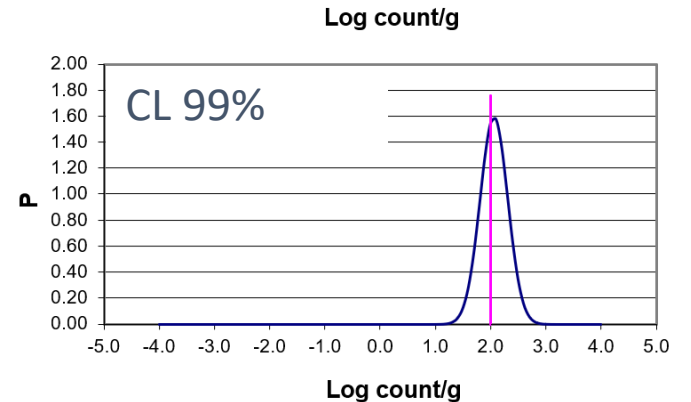
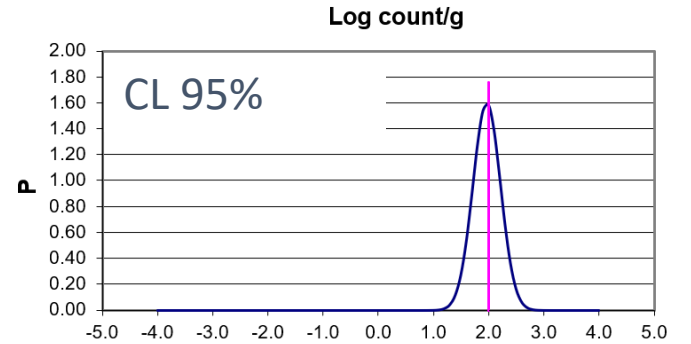
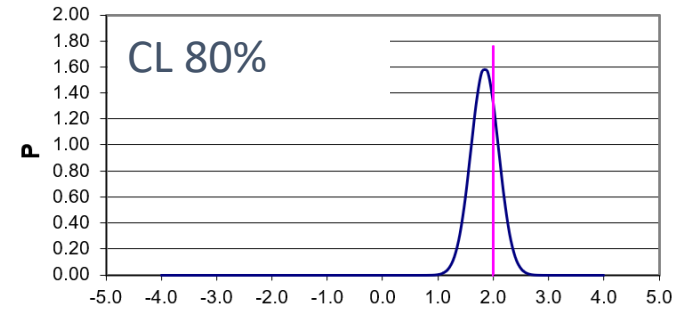
Confidence Level (%)	Arithmetic mean		Geometric mean	
	<i>cfu/g</i>	<i>log cfu/g</i>	<i>cfu/g</i>	<i>log cfu/g</i>
80.00	83.70	1.92	70.90	1.85
90.00	97.40	1.99	82.50	1.92
<b>95.00</b>	109.90	2.04	93.10	1.97
99.00	136.90	2.14	116.00	2.06
99.90	173.50	2.24	147.00	2.17



Limit  $m = 2 \log \text{cfu/g}$

Standard Deviation is 0.25 log cfu/g

Confidence Level (CL) represents P(reject)



# Changing confidence level and standard deviation

Sigma	Confidence	Arithmetic mean		Geometric mean			
		log cfu/g	Level (%)	cfu/g	log cfu/g	cfu/g	log cfu/g
0.25	80.00						
0.25	90.00						
0.25	95.00			109.90	2.04	93.10	1.97
0.80	80.00						
0.80	90.00						
0.80	95.00						
1.20	80.00						
1.20	90.00						
1.20	95.00						



Standard Deviation is 0.25 log cfu/g  
 Confidence Level (CL) represents P(reject)

Limit  $m = 2 \log \text{cfu/g}$

# Changing assumptions: confidence level

Sigma	Confidence Level (%)	Arithmetic mean		Geometric mean	
		cfu/g	log cfu/g	cfu/g	log cfu/g
0.25	80.00	83.70	1.92	70.90	1.85
	90.00	97.40	1.99	82.50	1.92
	95.00	109.90	2.04	93.10	1.97
0.80	80.00	181.60	2.26	33.30	1.52
	90.00	294.70	2.47	54.00	1.73
	95.00	434.30	2.64	79.60	1.90
1.20	80.00	873.70	2.94	19.20	1.28
	90.00	1805.50	3.26	39.70	1.60
	95.00	3230.80	3.51	71.00	1.85



Standard Deviation is 0.25 log cfu/g  
 Confidence Level (CL) represents P(reject)

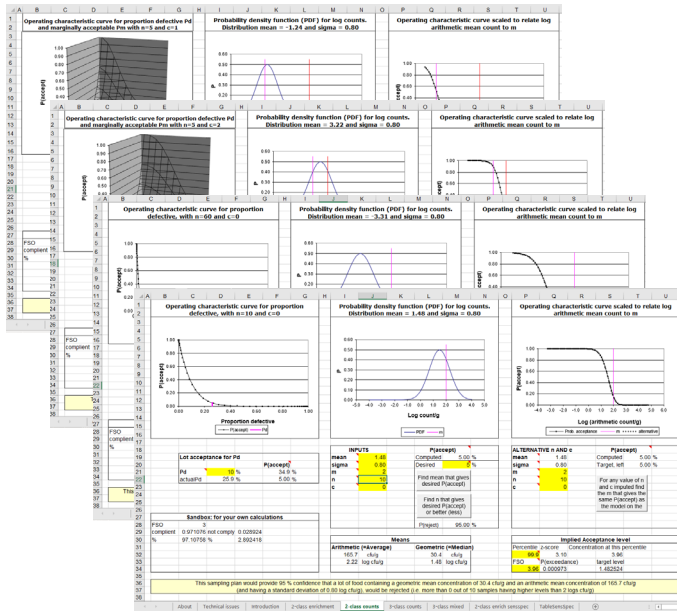
Limit  $m = 2 \log \text{cfu/g}$

# Summary

- The ICMSF sampling plan tool can be used to calculate and further interpret the performance of sampling plans included in Microbiological Criteria

- The robustness of key assumptions should be understood and choices should be well informed.

- See our other clips to understand how the ICMSF Sampling plan tool can be used to assess and interpret the performance of these MCs



For more information, see [www.icmsf.org](http://www.icmsf.org)