

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

## Part 3: MC for *Listeria monocytogenes* in RTE Foods supporting growth



Leon G.M. Gorris, PhD  
Food Safety Expert, The Netherlands  
*ICMSF Member 2001-present*

# Outline

- The Microbiological Criterion (MC) for Ready-to-Eat (RTE) foods that 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 supporting Lm growth\*

## Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can 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	Absence in 25 g (< 0.04 cfu/g) <sup>b</sup>	2 <sup>c</sup>

<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> Absence in a 25-g analytical unit. This criterion is based on the use of ISO 11290-1 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 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes*.

Such a lot may consist of 55% of the 25g samples being negative and up to 45% of the 25 g samples being positive. 0.5 % of this lot could harbour concentrations above 0.1 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.

# MC: RTE foods supporting Lm growth

## Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can occur

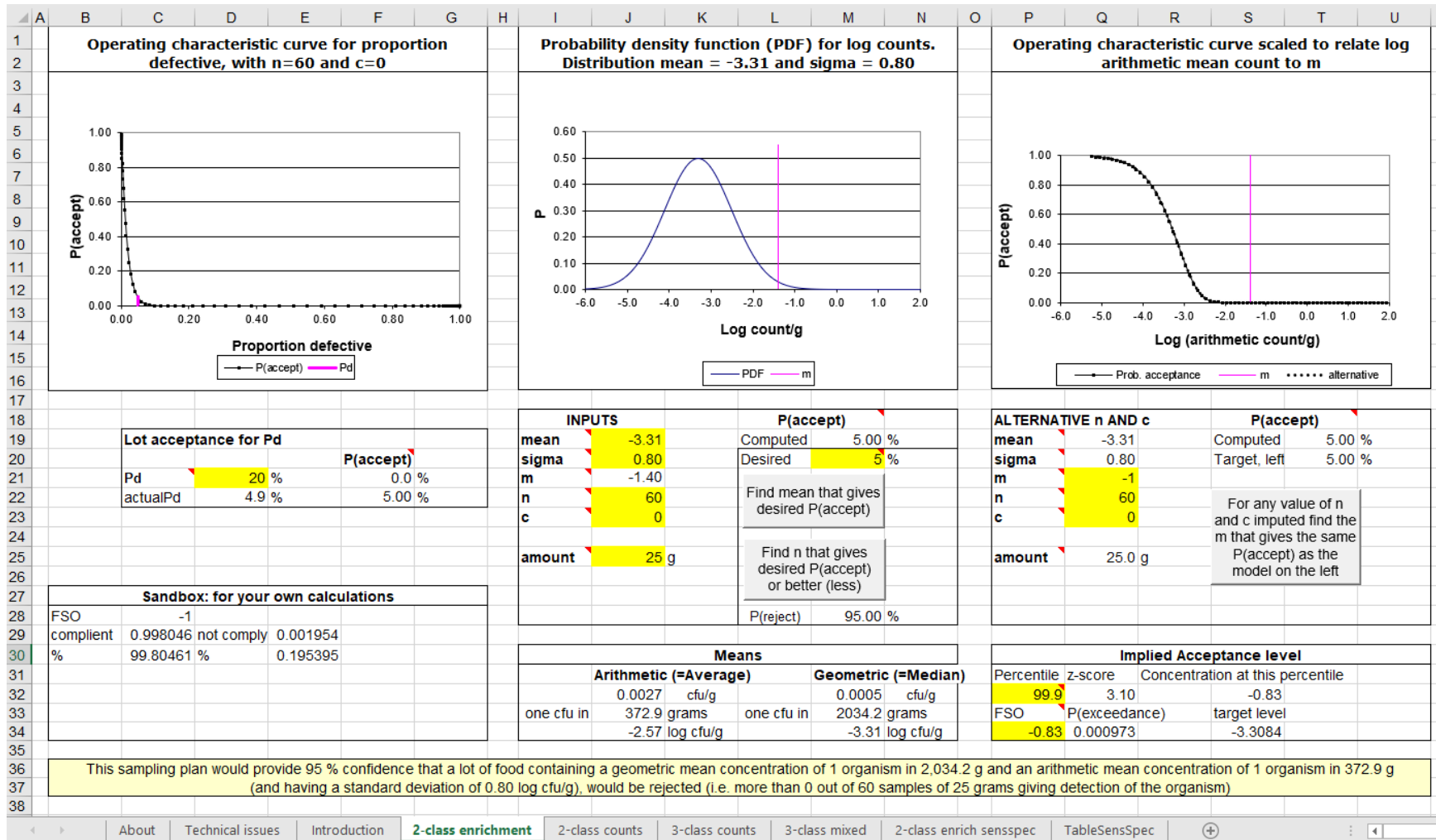
Point of application	Microorganism	<i>n</i>	<i>c</i>	<i>m</i>	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	<i>Listeria monocytogenes</i>	5 <sup>a</sup>	0	Absence in 25 g (< 0.04 cfu/g) <sup>b</sup>	2 <sup>c</sup>

<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 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes*.

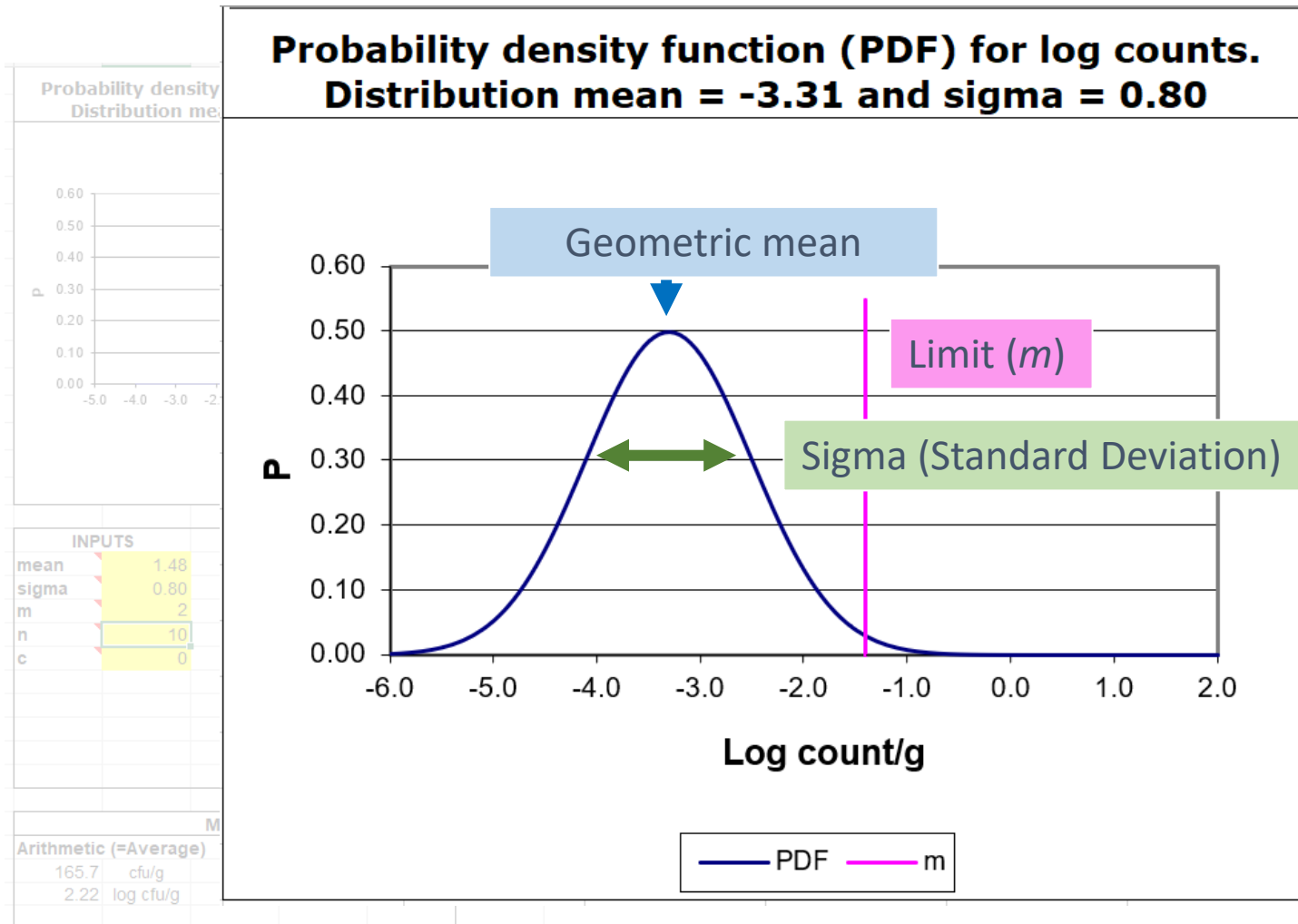
Such a lot may consist of 55% of the 25g samples being negative and up to 45% of the 25 g samples being positive. 0.5 % of this lot could harbour concentrations above 0.1 cfu/g.

# The 2-class enrichment dashboard

2-class enrichment



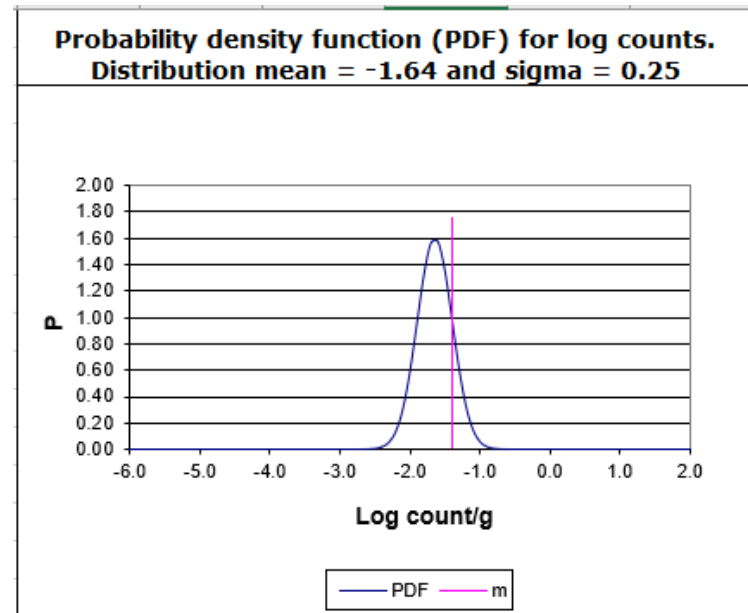
# The 2-class enrichment dashboard



# The 2-class enrichment dashboard

INPUTS			P(accept)		
mean	-1.94		Computed	10.00	%
sigma	0.80		Desired	10	%
m	-1.40		Find mean that gives desired P(accept)		
n	5				
c	0		Find n that gives desired P(accept) or better (less)		
amount	25	g			
			P(reject)	90.00	%
Means					
	Arithmetic (=Average)			Geometric (=Median)	
	0.0624	cfu/g		0.0114	cfu/g
one cfu in	16.0	grams	one cfu in	87.5	grams
	-1.21	log cfu/g		-1.94	log cfu/g

# The Lm MC in the 2-class enrichment dashboard



Standard deviation

$M$ ,  $n$ ,  $c$  and amount from MC sampling plan

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	Find n that gives desired P(accept) or better (less)	
c	0		
amount	25 g	P(reject)	95.00 %
Means			
Arithmetic (=Average)		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

Calculated mean and distribution

Chance to accept a non-compliant lot

Chance to reject a non-compliant lot



# The Lm MC in the 2-class enrichment dashboard

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)	
		P(reject)	95.00 %
Means			
Arithmetic (=Average)		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

This sampling plan would provide 95 % confidence that a lot of food containing a geometric mean concentration of 1 organism in 44.1 g and an arithmetic mean concentration of 1 organism in 37.4 g (and having a standard deviation of 0.25 log cfu/g), would be rejected (i.e. more than 0 out of 5 samples of 25 grams giving detection of the organism)

This sampling plan would provide

- 95% confidence that a lot of food containing a
- geometric mean concentration of 1 organism in 44.1 g and an
- arithmetic mean concentration of 1 organism in 37.4 g
- (and having a standard deviation of 0.25 log cfu/g),
- would be rejected
- (i.e. more than 0 out of 5 samples in 25 g giving detection of the organism)

# MC: RTE foods supporting Lm growth

## Microbiological criteria for ready-to-eat foods in which growth of *L. monocytogenes* can 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	Absence in 25 g (< 0.04 cfu/g) <sup>b</sup>	2 <sup>c</sup>

<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 0.023 cfu/g and an analytical standard deviation of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes*.

Such a lot may consist of 55% of the 25g samples being negative and up to 45% of the 25 g samples being positive. 0.5 % of this lot could harbour concentrations above 0.1 cfu/g.

# MC: RTE foods supporting Lm growth

INPUTS			P(accept)		ALTERNATIVE n AND c			P(accept)	
mean	-1.64		Computed	5.00 %	mean	-1.64		Computed	5.00 %
sigma	0.25		Desired	5 %	sigma	0.25		Target, left	5.00 %
m	-1.40		Find mean that gives desired P(accept)		m	-1		For any value of n and c imputed find the m that gives the same P(accept) as the model on the left	
n	5				n	5			
c	0				c	0			
amount	25 g		Find n that gives desired P(accept) or better (less)		amount	25.0 g			
			P(reject)	95.00 %					
Means					Implied Acceptance level				
Arithmetic (=Average)			Geometric (=Median)		Percentile	z-score	Concentration at this percentile		
one cfu in	0.0267	cfu/g	one cfu in	0.0227	99.9	3.10	-0.87		
	37.4	grams		44.1	FSO	P(exceedance)	target level		
	-1.57	log cfu/g		-1.64	-1.00	0.004946	-1.7746		

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



FSO	P(exceedance)
-1.00	0.004946

Enter concentration in log cfu/g

Means that proportion over limit is 0.5%

# MC performance: Changing assumptions

**Microbiological criteria for ready-to-eat foods  
in which growth of *L. monocytogenes* can occur**

$n$	$c$	$m$	Class Plan
$5^a$	0	Absence in 25 g ( $< 0.04$ 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)

Sigma  
(log cfu/g)

0.25

0.80

1.20



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)	
		P(reject)	95.00 %
Means			
Arithmetic (=Average)		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

Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Changing assumptions: standard deviation (SD)

Sigma  
(log cfu/g)

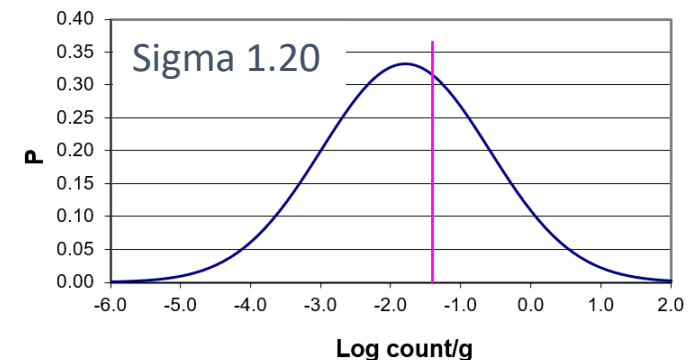
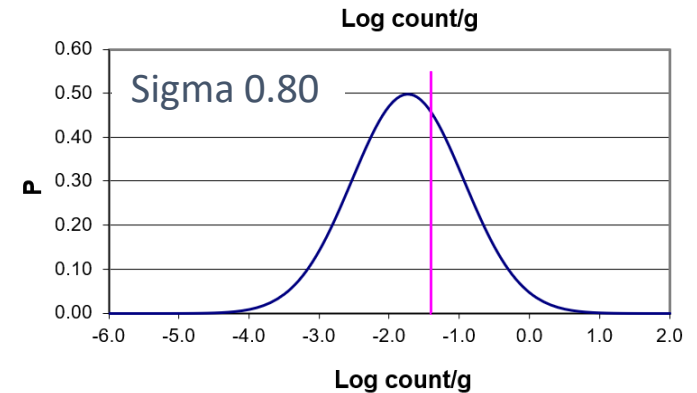
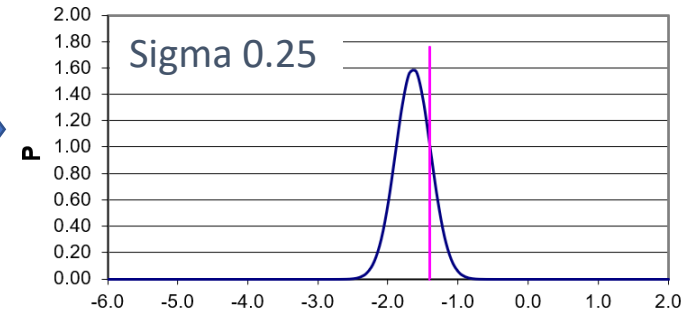
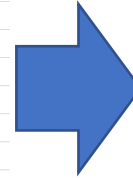
0.25

0.80

1.20



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	Find n that gives desired P(accept) or better (less)	
amount	25 g		
		P(reject)	95.00 %
Means			
Arithmetic (=Average)		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



Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Changing assumptions: standard deviation (SD)



	Arithmetic mean		Geometric mean	
Sigma	1 cfu/x g	log cfu/g	1 cfu/x g	log cfu/g
0.25	37.4	-1.61	4.1	-1.64
0.80	10	-1.00	54.7	-1.74
1.20	1.4	-0.14	62.3	-1.79


Limit  $m = -1.4 \log \text{cfu/g}$

Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# Impact of sigma on lot status

Calculate using P(exceedance)



Sigma (log cfu/g)	Lm levels in proportions (%) of a food lot		
	Negative	Positive	> 0.1 cfu/g
<b>0.25</b>	55 %	45 %	0.5 %
<b>0.80</b>	55 %	45 %	17.8 %
<b>1.20</b>	55 %	45 %	24.4 %

Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)



# Changing assumptions: confidence level

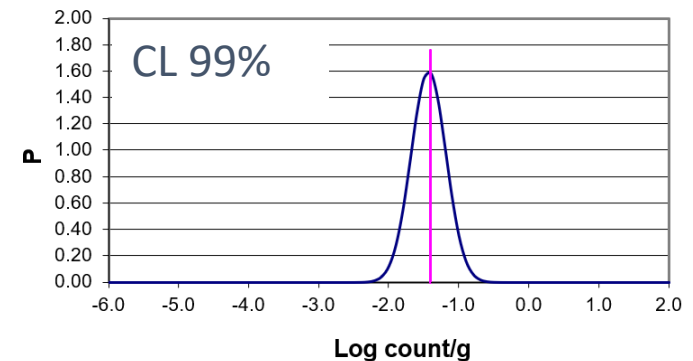
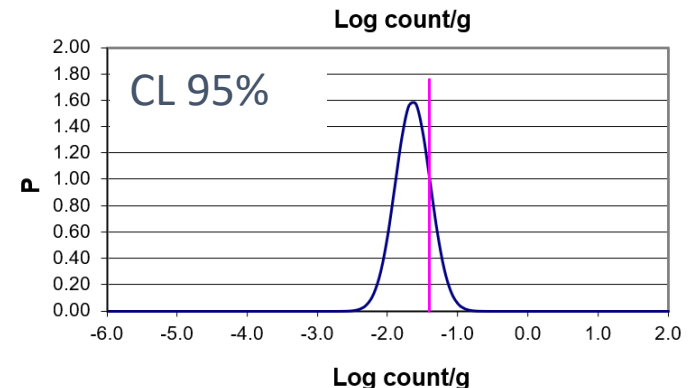
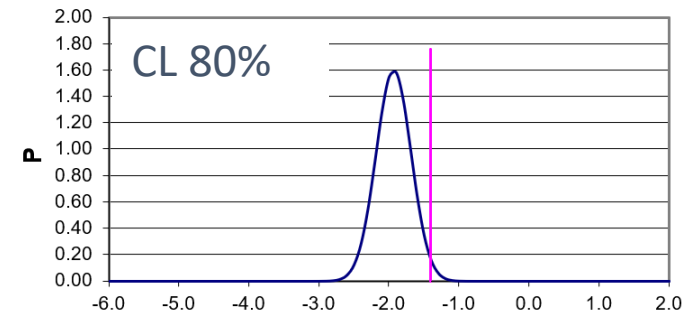
Confidence	Arithmetic mean		Geometric mean	
Level (%)	1 cfu/x g	log cfu/g	1 cfu/x g	log cfu/g
80.0	73.10	-1.86	86.30	-1.94
90.0	49.80	-1.70	58.80	-1.77
<b>95.0</b>	37.40	-1.57	44.10	-1.64
99.0	23.10	-1.36	27.20	-1.43
99.9	14.30	-1.16	16.90	-1.23



Limit  $m = -1.4 \log \text{cfu/g}$

Standard Deviation is 0.25 log cfu/g

Confidence Level (CL) represents  $P(\text{reject})$



# 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				
Codex	0.25	95.00	37.40	-1.57	44.10	-1.64
	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  $m = -1.4 \log \text{cfu/g}$

# Changing confidence level and standard deviation



Sigma	Confidence	Arithmetic mean		Geometric mean	
		cfu/g	log cfu/g	cfu/g	log cfu/g
0.25	80.00	73.10	-1.86	86.30	-1.94
0.25	90.00	49.80	-1.70	58.80	-1.77
0.25	95.00	37.40	-1.57	44.10	-1.64
0.80	80.00	29.10	-1.46	158.60	-2.20
0.80	90.00	16.00	-1.21	87.50	-1.94
0.80	95.00	10.00	-1.00	54.70	-1.74
1.20	80.00	5.80	-0.76	264.50	-2.42
1.20	90.00	2.60	-0.42	118.30	-2.07
1.20	95.00	1.40	-0.14	62.30	-1.79

Standard Deviation is 0.25 log cfu/g

Confidence Level (CL) represents P(reject)

Limit  $m = -1.4 \log \text{cfu/g}$

# Impact of sigma on lot status



<b>Sigma</b> log cfu/g	<b>Confidence Level (%)</b>	<b>Proportion of a food lot over 0.1 cfu/g (%)*</b>
0.25	90.0	0.10
0.25	95.0	0.50
0.80	90.0	12.0
0.80	95.0	17.8
1.20	90.0	18.6
1.20	95.0	25.4

\* Calculated using P(exceedance)

Confidence Level (CL) is 95%

Sigma represents Standard Deviation (SD)

# 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

