

USP Open Forum

# Comparing Probiotic Plate Count Methods

June 16, 2022, 10:00 am • 12:30 pm ET

Virtual Meeting



# USP Approach to Probiotic Enumeration

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Probiotic Expert Committee Member



# Zooming in on Probiotics



## Product

<b>Supplement Facts</b>			
Serving Size: One (1) Packet			
	Amount Per Serving	% Daily Value ages 1-3	% Daily Value 4+ years of age
Calories	5		
Total Carbohydrate	1 g	<1% <sup>++</sup>	<1% <sup>+</sup>
Total Sugars	0 g	**	**
Incl. 0g Added Sugars		0% <sup>++</sup>	0% <sup>+</sup>
Vitamin D (as cholecalciferol)	10 mcg (400 IU)	67%	50%
Proprietary Blend Total Cultures	18 mg (3.5 billion CFUs)		
<i>Lactobacillus rhamnosus</i> GG (LGG <sup>®</sup> )		**	**
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> (BB-12 <sup>®</sup> )		**	**
2'-Fucosyllactose	1 g	**	**

<sup>++</sup> Percent Daily Values are based on a 1,000 calorie diet.  
<sup>+</sup> Percent Daily Values are based on a 2,000 calorie diet  
<sup>\*\*</sup> Daily Value not established.

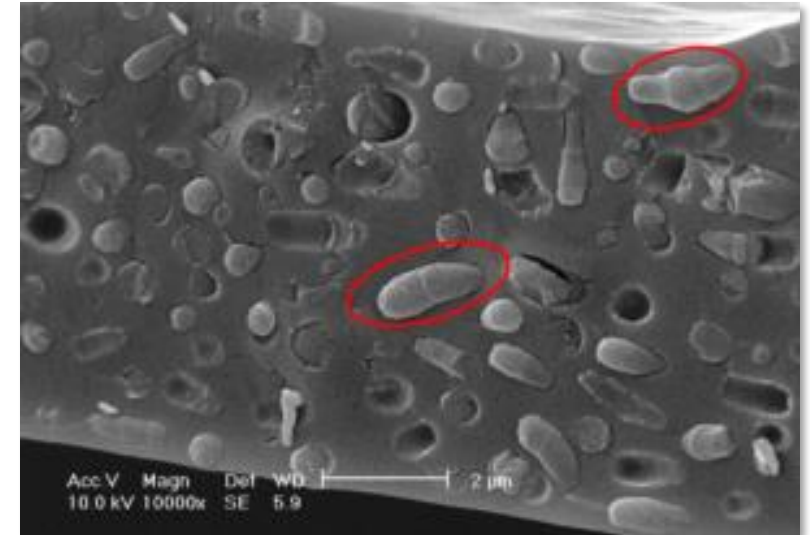
Other Ingredients: Maltodextrin  
May contain traces of milk (lactose).

## Content



Blend of lyophilized probiotic bacteria and excipients

## Dietary ingredient



Viable lyophilized bacterial cells

Photo from ISAPP  
<https://isappscience.org/tag/freeze-drying/>

# Probiotic potency



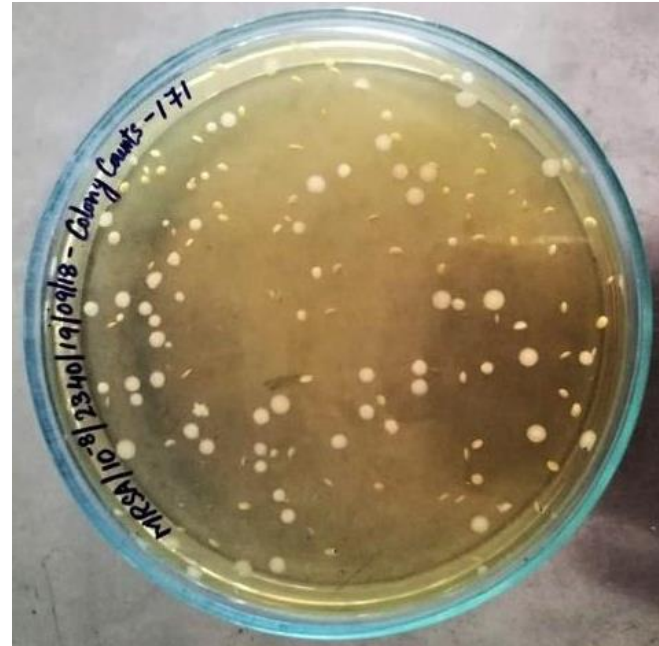
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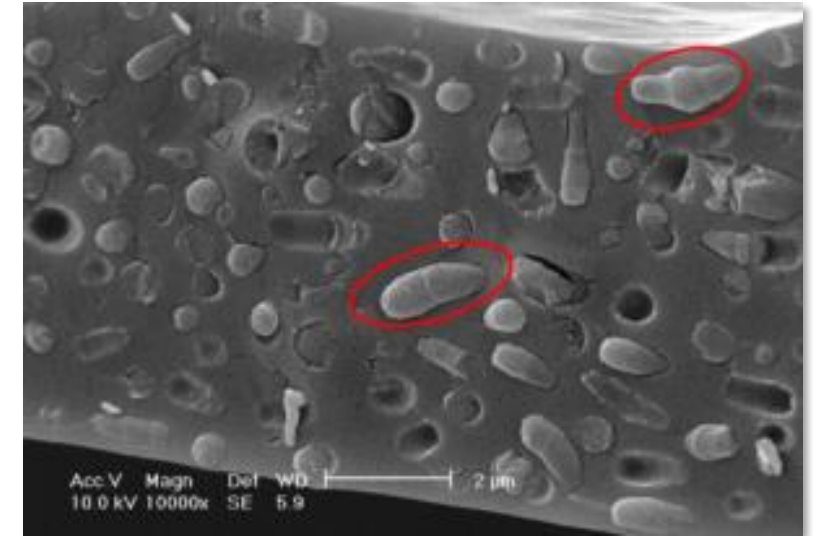
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## Colony Forming Units (CFU) on agar plate



## Enumeration of viable cells



Viable cell

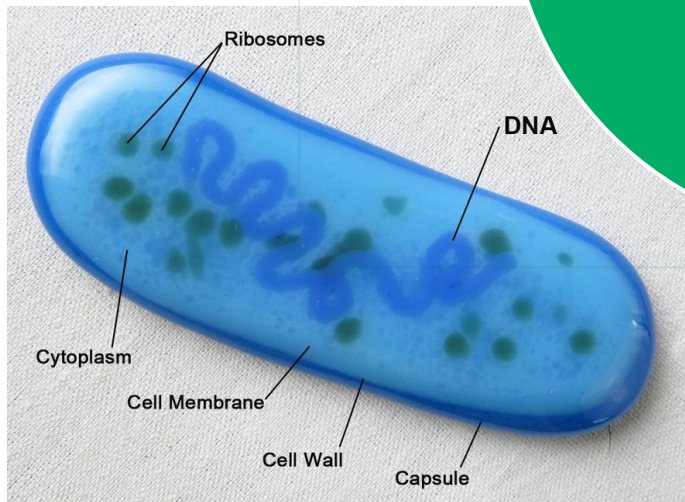
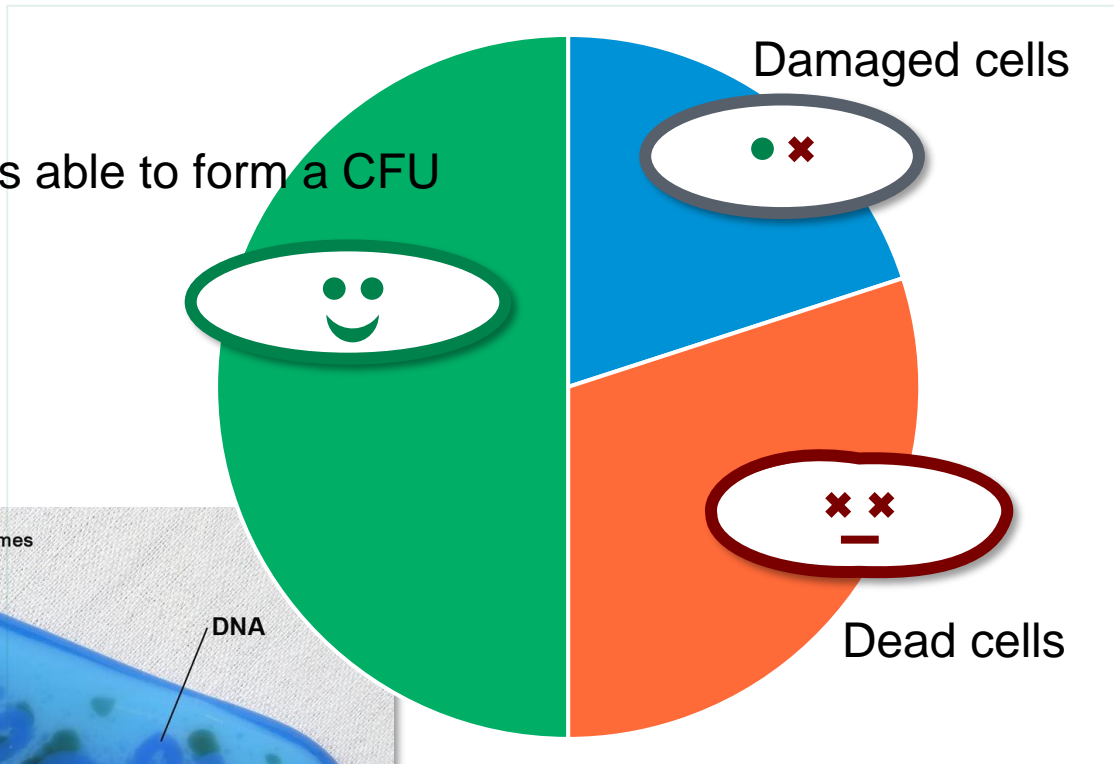


Dead cell

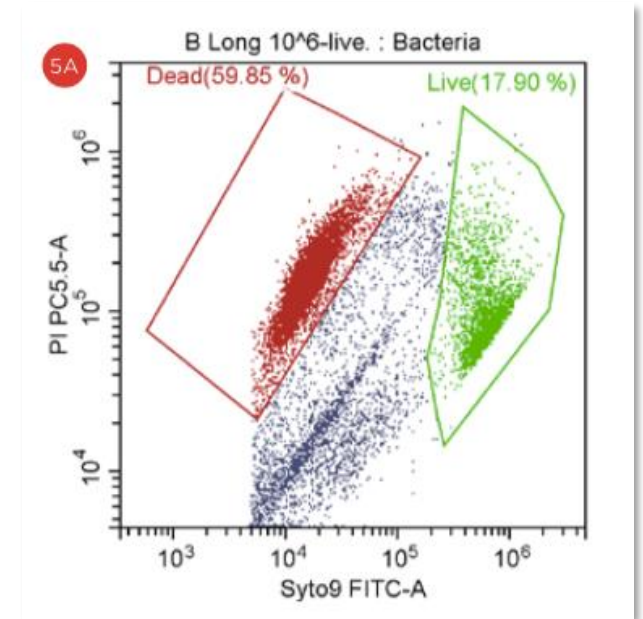
# Can all bacterial cells form a CFU?



Viable cells able to form a CFU

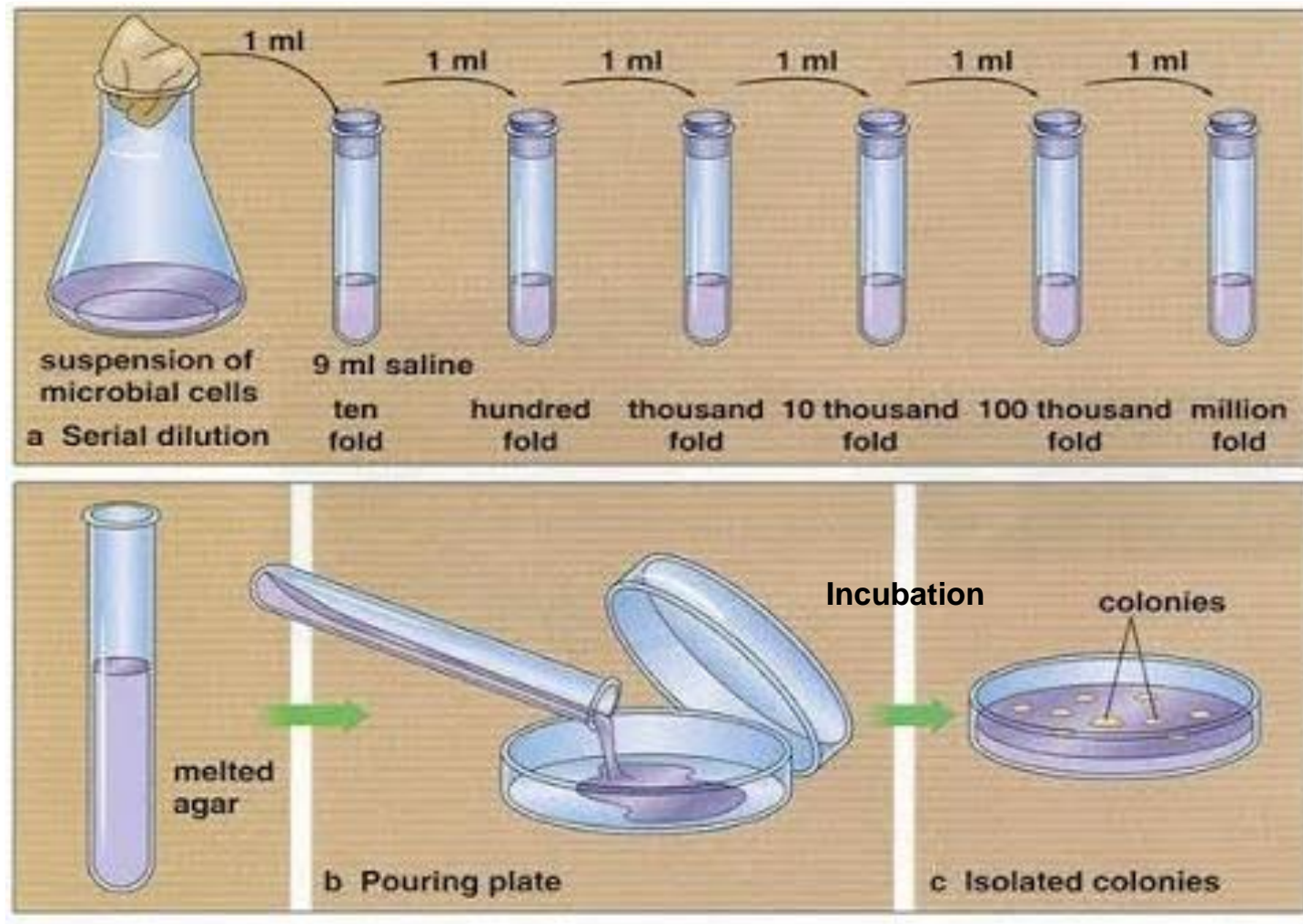


Membrane intact cells measured by flow cytometry



**Correlation to CFU dosage used in clinical trials**

# CFU: Golden standard for probiotic potency



# Plate count methods in USP



Chapter/Monograph	Plate count method	Status
Probiotic Tests <64>	Enumeration assay for non-spore-forming bacteria strains	Official
<i>Lactobacillus rhamnosus</i>	<64>	Official
<i>Lactobacillus reuteri</i>	<64>	Official
<i>Lactobacillus acidophilus</i>	<64>	01Aug2022
<i>Lactobacillus paracasei</i>	<64>	01Aug2022
<i>Lacticaseibacillus casei</i>	<64>	01Aug2022
<i>Bifidobacterium bifidum</i>	<64> modified (agar)	Official
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	<64> modified (agar, incubation)	Official
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	<64> modified (agar)	Official
<i>Bifidobacterium longum</i> subsp. <i>infantis</i>	<64> modified (agar, sample preparation)	01Aug2022
<i>Bacillus coagulans</i>	Specific method for <i>Bacillus coagulans</i>	Official
<i>Bacillus clausii</i>	Specific method for <i>Bacillus clausii</i>	Official
Additional monographs under way	Unknown	Pending

# General challenges of plate count methods



Ideal sample preparation

Type and shelf life of agar

Enumeration of multiple strains with different growth requirements

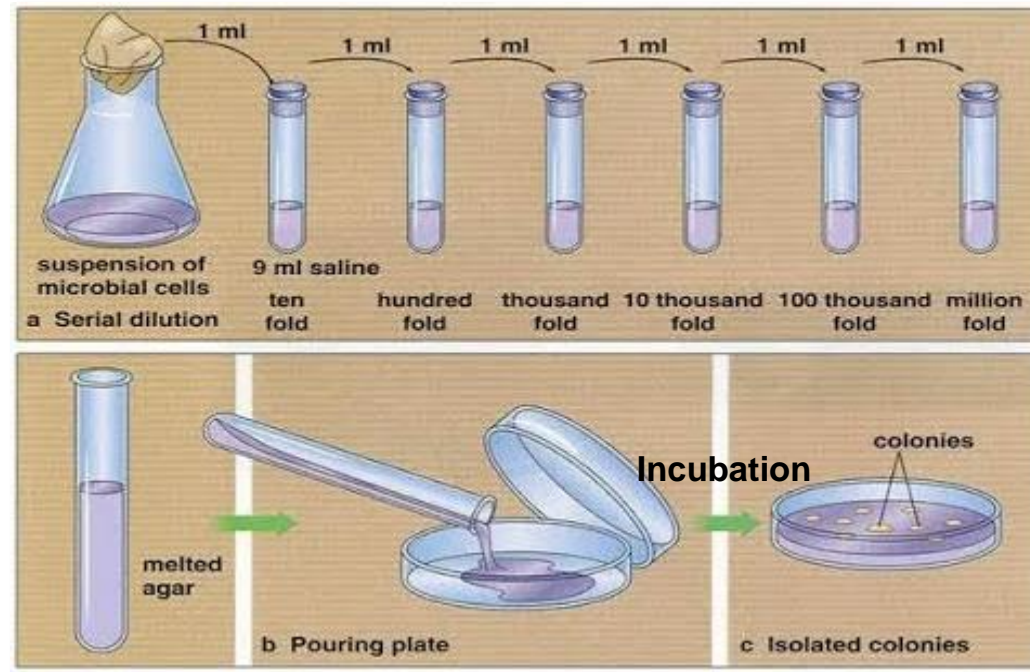
Time consuming - up to 6 days

Robustness of method

No reference standards

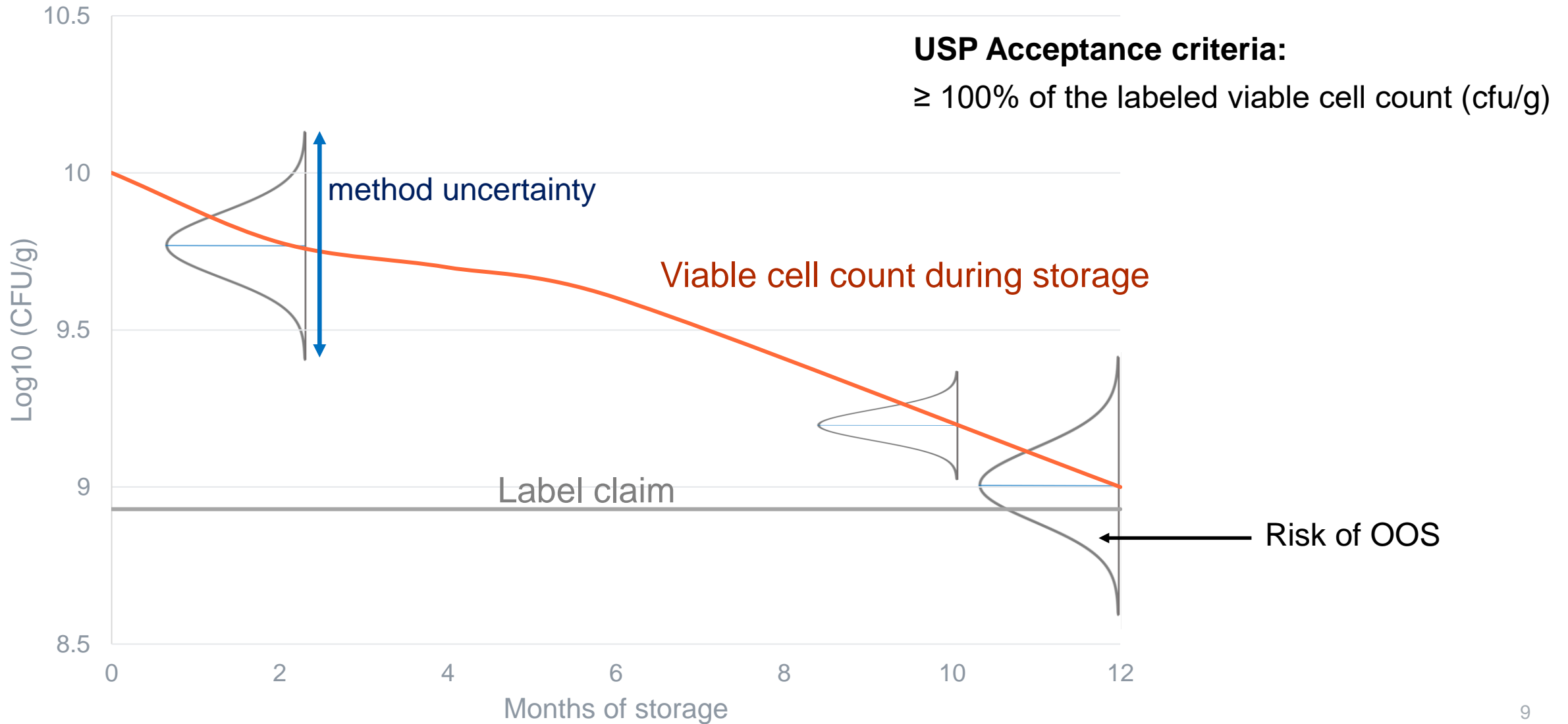
Uncertainty of method

Comparison of methods





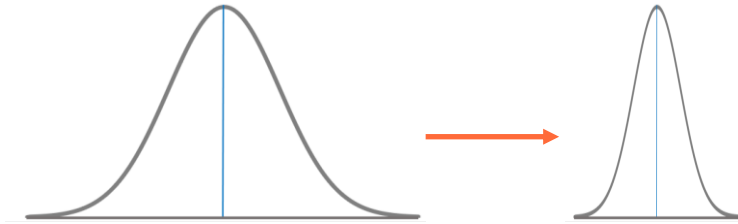
# Impact of method uncertainty



# How to manage CFU methods

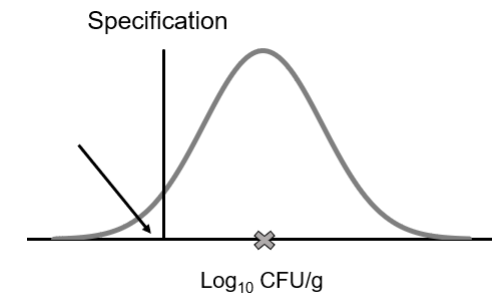


How to evaluate and control the method uncertainty?



How to compare the performance of two different methods?

How to evaluate the risk of a false OOS?



How to evaluate the impact on changing to a new supplier of agar?

How many replicates are needed?

# Thank You



**The standard of trust**

# Stay Connected

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