

# Spore concerns and testing in dairy: Reducing variability and establishing standards

---

NICOLE MARTIN (NHW6@CORNELL.EDU)

MILK QUALITY IMPROVEMENT PROGRAM

DEPARTMENT OF FOOD SCIENCE, CORNELL UNIVERSITY



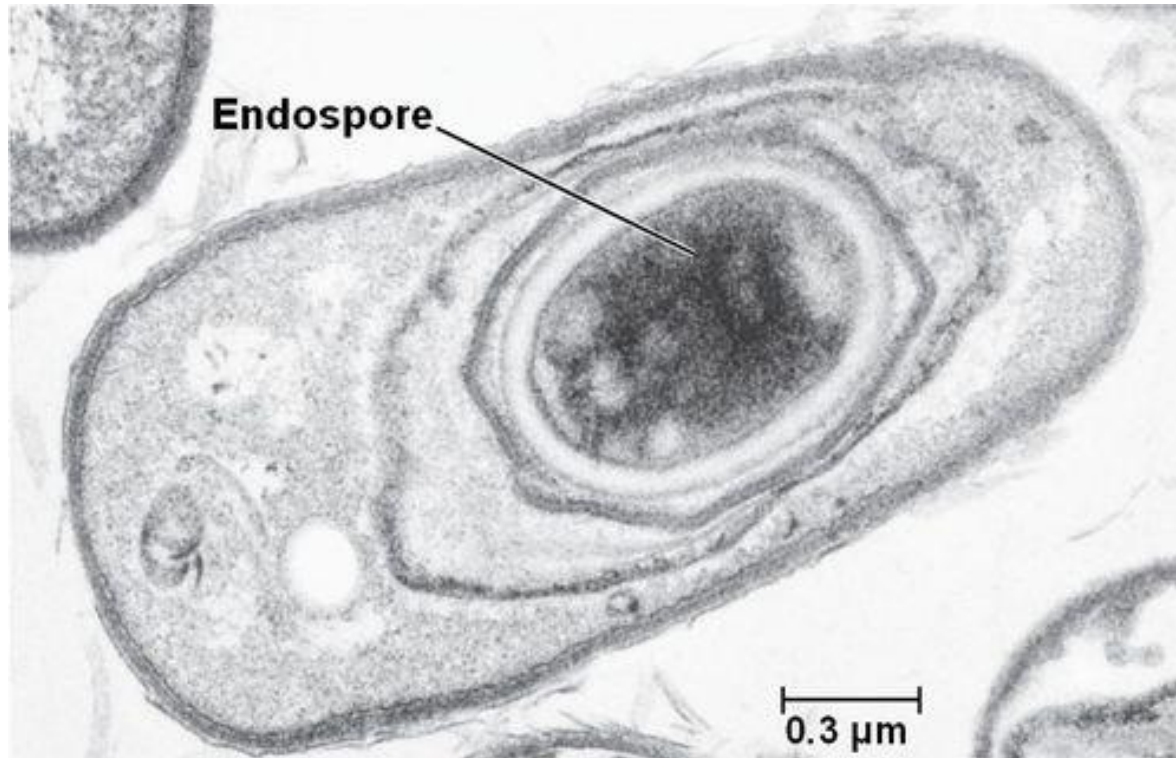
# Overview

---

- What are they and why do we care
- Raw to finished product contaminants
- Testing methods matter

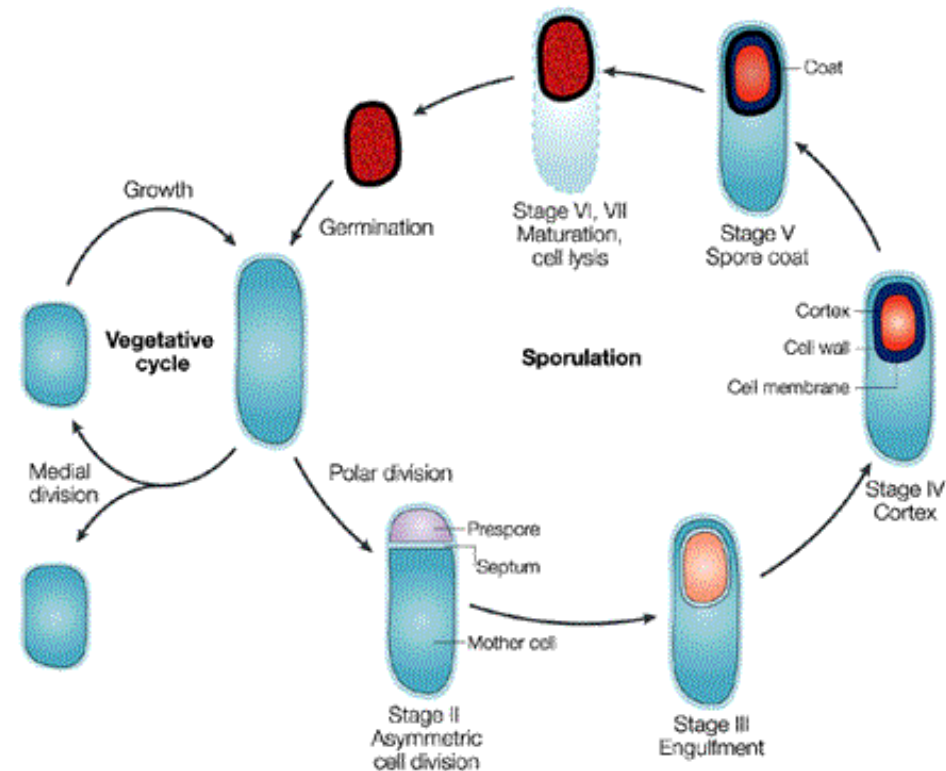
# Spores: Defense strategies for harsh environmental conditions

---



- Endospores are formed under stressful environmental conditions
- Allow bacteria to persist in environments that would otherwise be lethal
  - **Heat (pasteurization)**
  - Drying (powders)
  - Radiation
  - Sanitizers
- Under favorable conditions, spores return to a metabolically active state

# Sporulation and germination – The key to the spore challenge in dairy foods

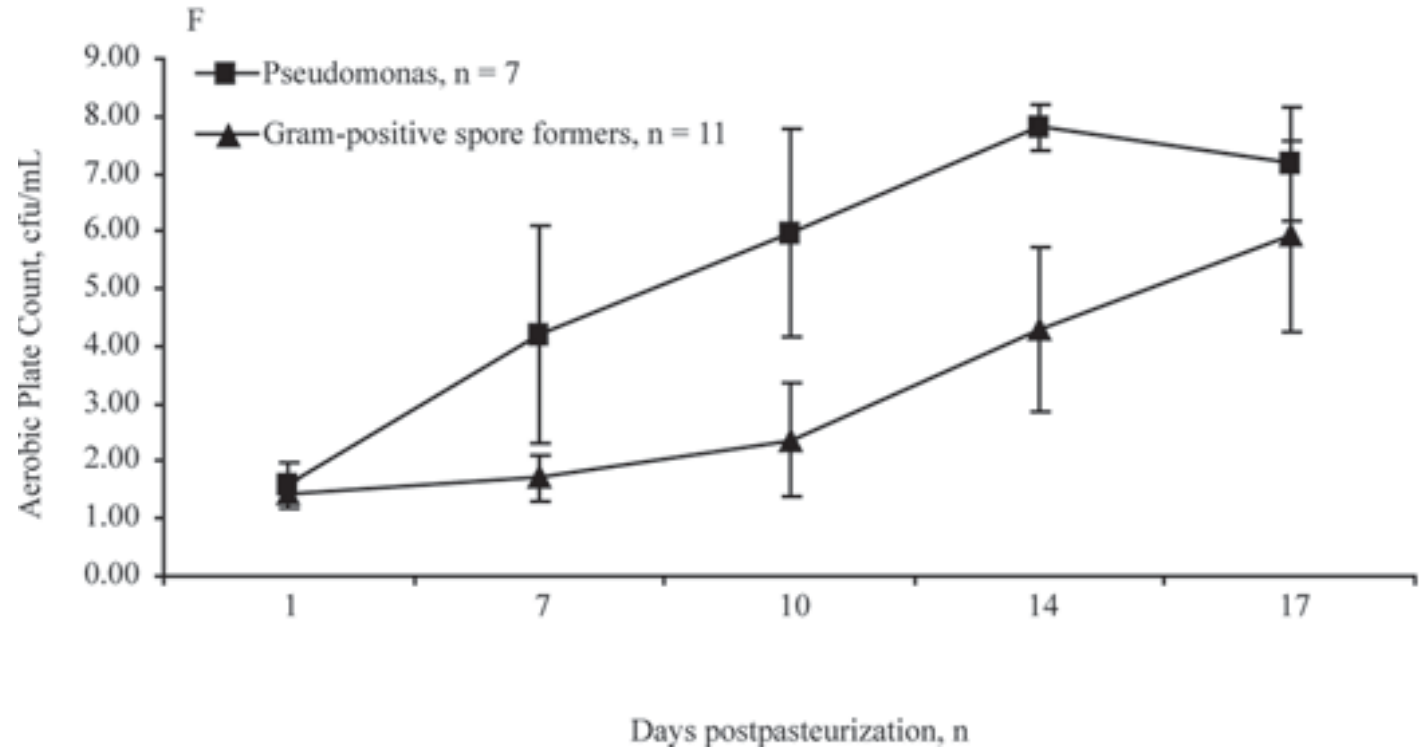


# Dairy relevant sporeforming bacteria



## Fluid Milk

- **Psychrotolerant Sporeformers**
  - *Paenibacillus*
  - *Viridibacillus*
  - *Bacillus weihenstephanensis*



# Dairy relevant sporeforming bacteria



## Cheese

- Anaerobic Butyric Acid Bacteria (BAB)
  - *Clostridium tyrobutyricum*
  - *C. butyricum*
  - *C. sporogenes*



- Historical association with high levels of BAB in silage – raw milk used for certain cheeses in Switzerland cannot come from cows fed silage
- Penalties used to regulate BAB in raw milk used for cheesemaking in the Netherlands

# Dairy relevant sporeforming bacteria



## Dairy Powder

- Mesophilic and Thermophilic Sporeformers
  - *Bacillus licheniformis*
  - *Geobacillus*
  - *Anoxybacillus*

**Table 1.** Typical example of Spore-Formers Specifications-Infant Formula for Export

Contaminant	Range of limit (CFU)
Mesophilic plate count	Max : <5,000 to 10,000/g
Thermophilic plate count	Max: <5,000/g
Aerobic spore, mesophilic	<500 to <1,000/g
Aerobic spore, thermophilic	<500 to <1,000/g
<i>Bacillus Cereus</i>	Max: <100/g
<i>Clostridium perfringens</i>	Max: negative /0.1g
Sulfite reducing <i>clostridia</i>	Max: <10 to <25/g

Source: USDEC

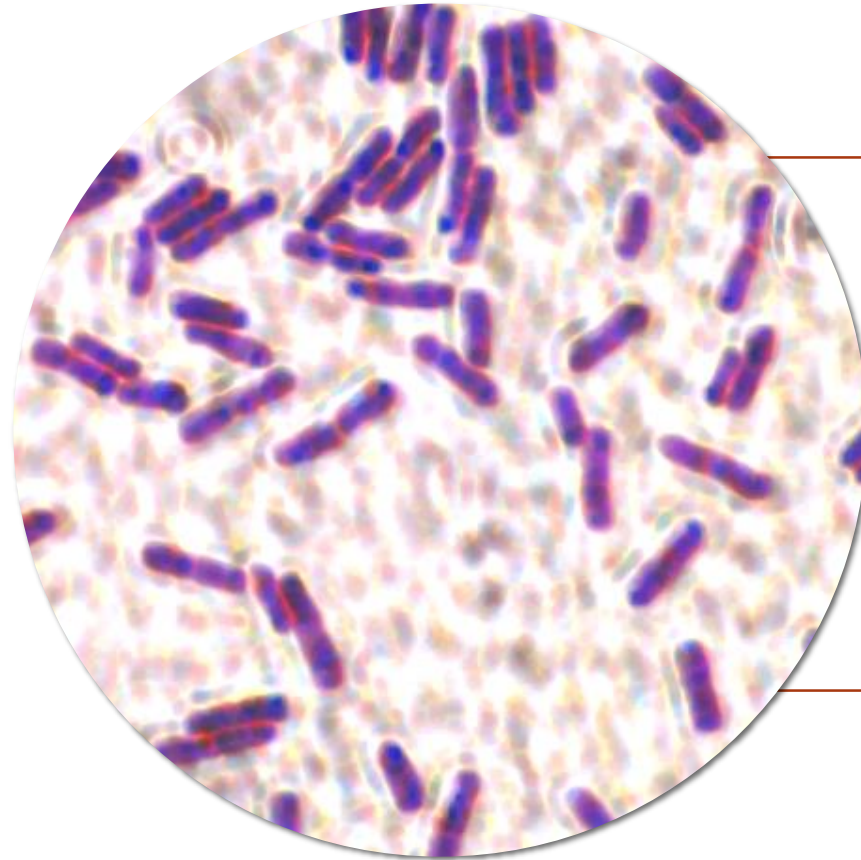
**Table 2.** Typical example of Spore-Formers-Recombined and UHT for Export

Contaminant	Limit (CFU/g)
Mesophilic plate count	<10,000
Thermophilic plate count	<5,000 to <10,000
Aerobic spore, thermophilic	<500 to 2,000
Thermoresistant spore count	<500

Source: USDEC

# Sporeforming bacteria- Farm to finished product contaminants

---



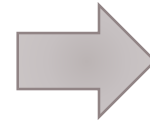
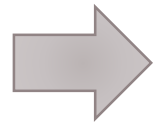
# Sporeforming bacteria in dairy products

---

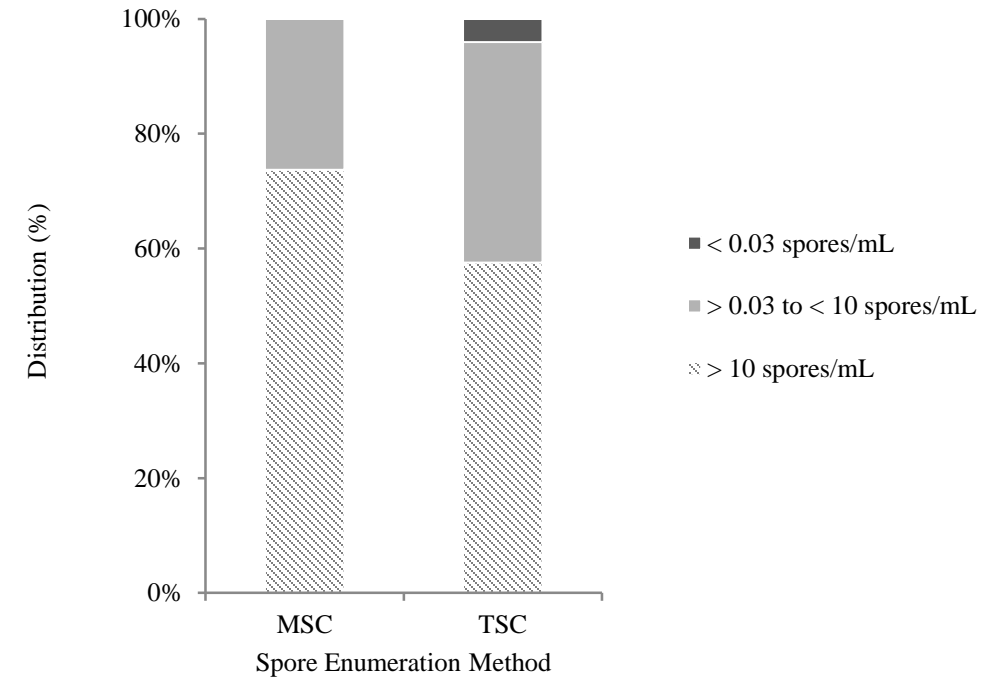
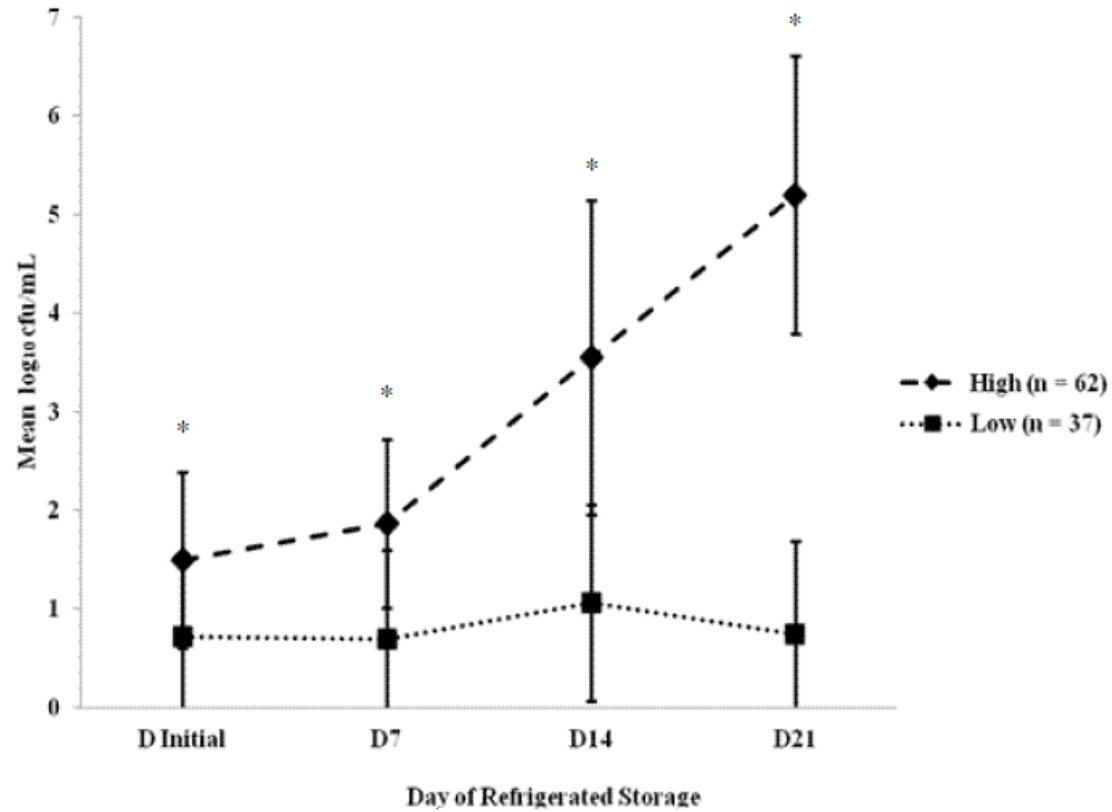


# Sporeforming bacteria in dairy products

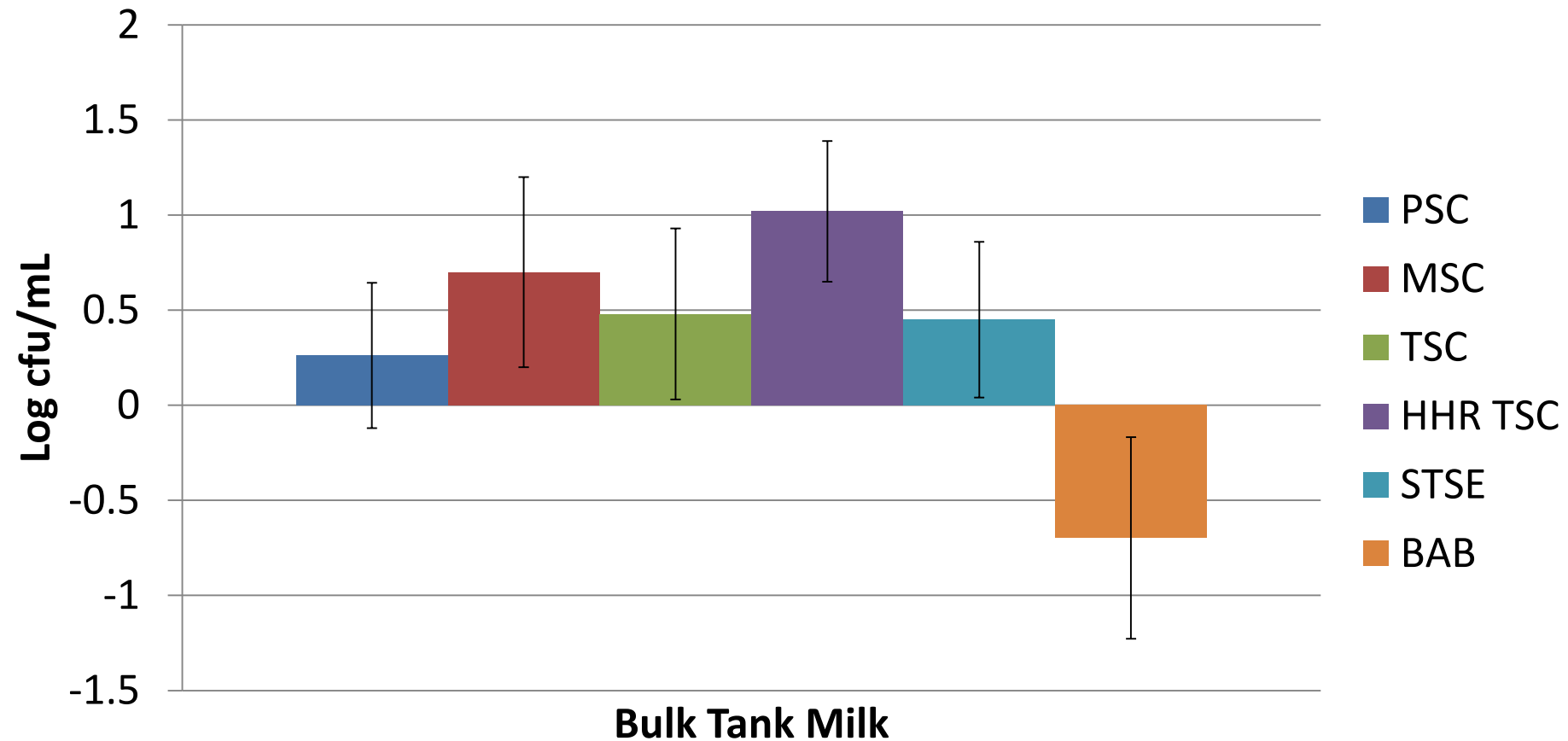
---



# Mesophilic spores represent the highest proportion of sporeforming bacteria in raw milk

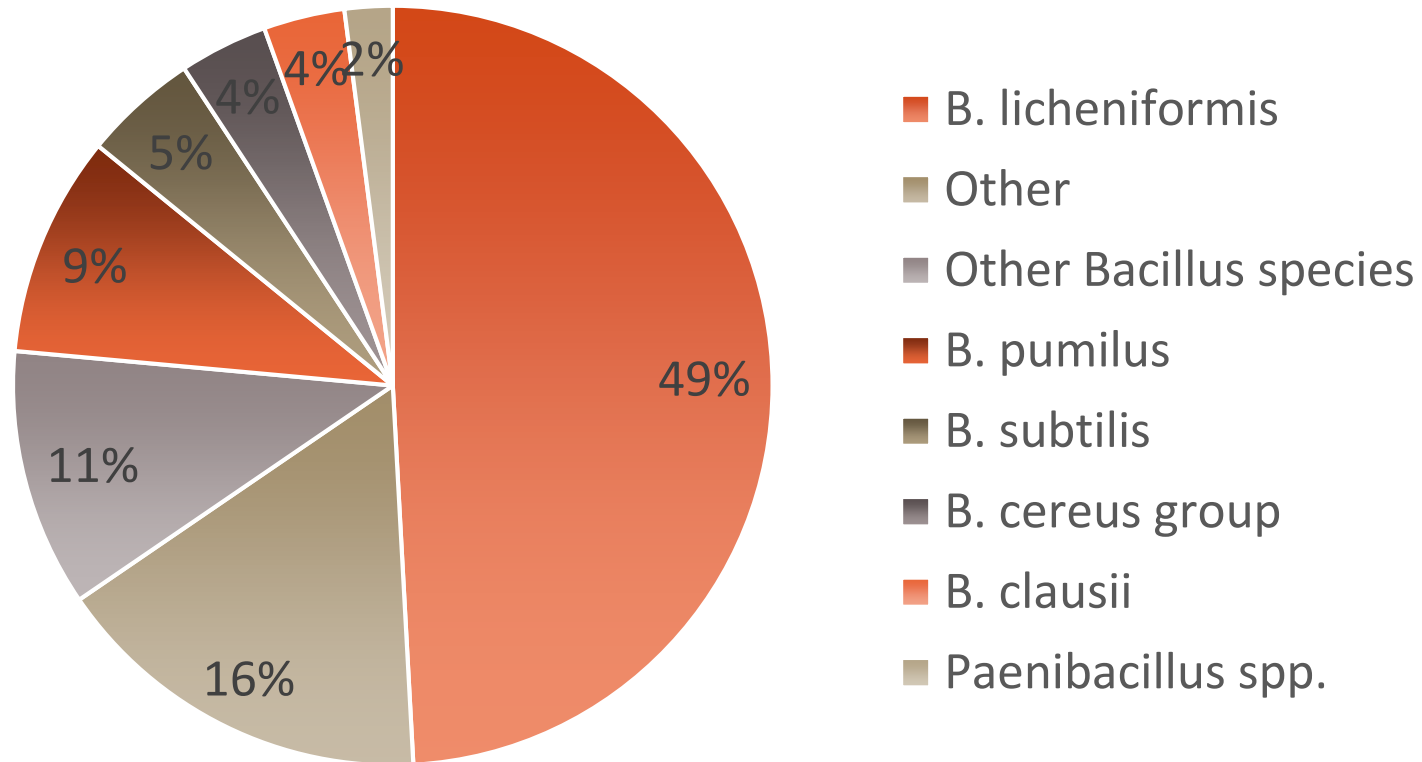


# Spores are present in low levels in raw milk



# *Bacillus licheniformis* accounts for nearly half of spores in bulk tank raw milk

---



# Sources of sporeforming bacteria

---



- Soil
- Feed
- Bedding

# Sources of sporeforming bacteria

---



- Manure
- Drinking water

# Sources of sporeforming bacteria

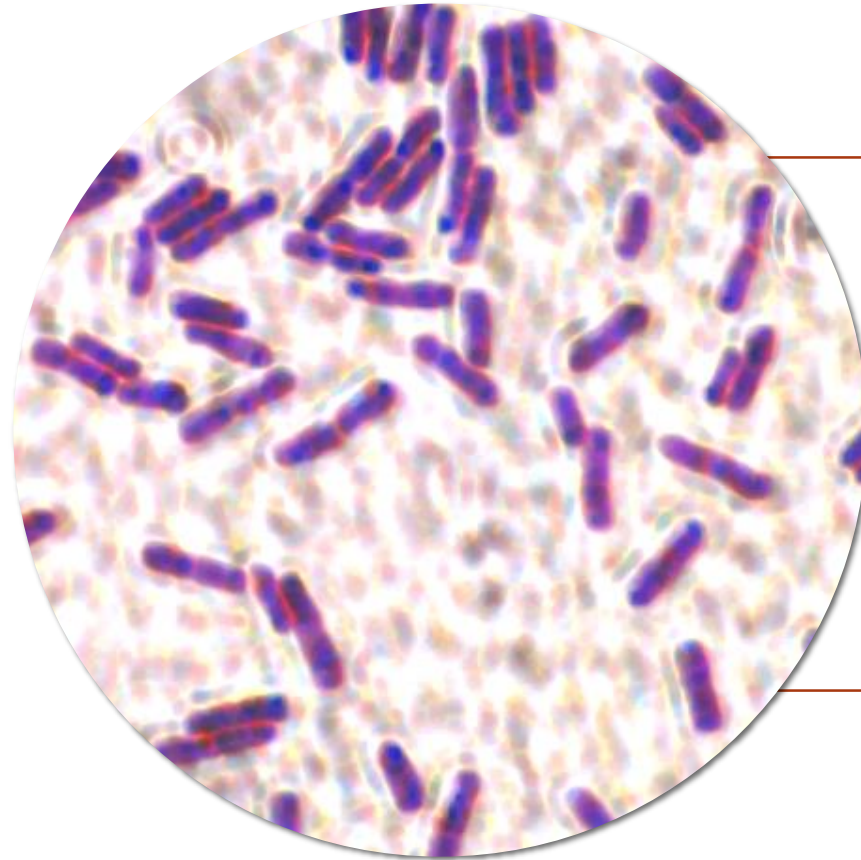
---



- Air
- Equipment swab
- Filter sock
- Individual cow milk
- Teat swab
- Towel
- Wash water
- Bulk tank milk

# Sporeforming bacteria- Farm to finished product contaminants

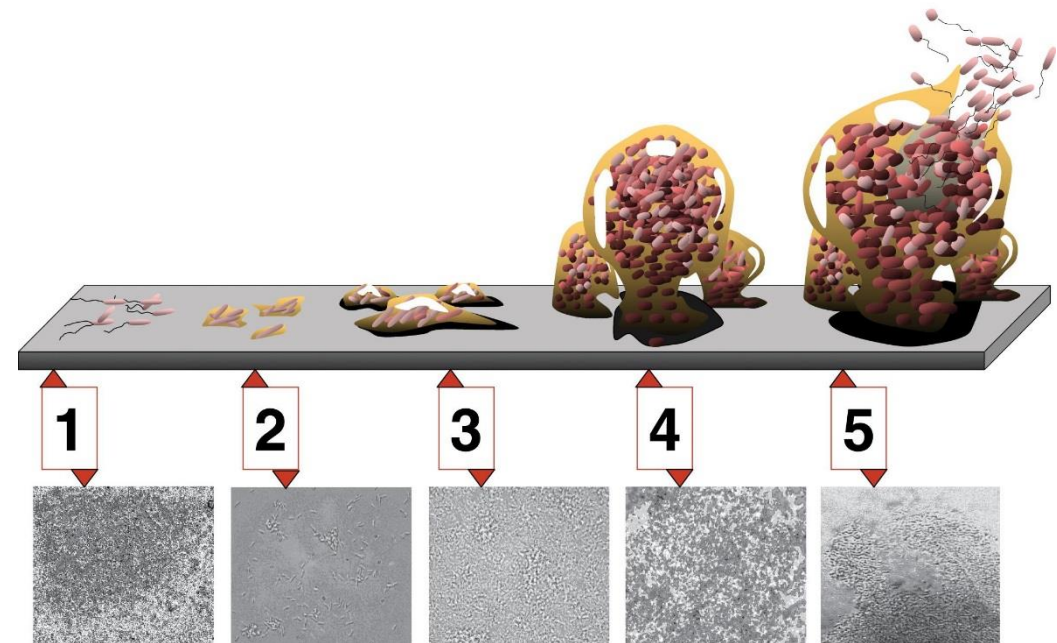
---



# Sporeforming bacteria in processing environments

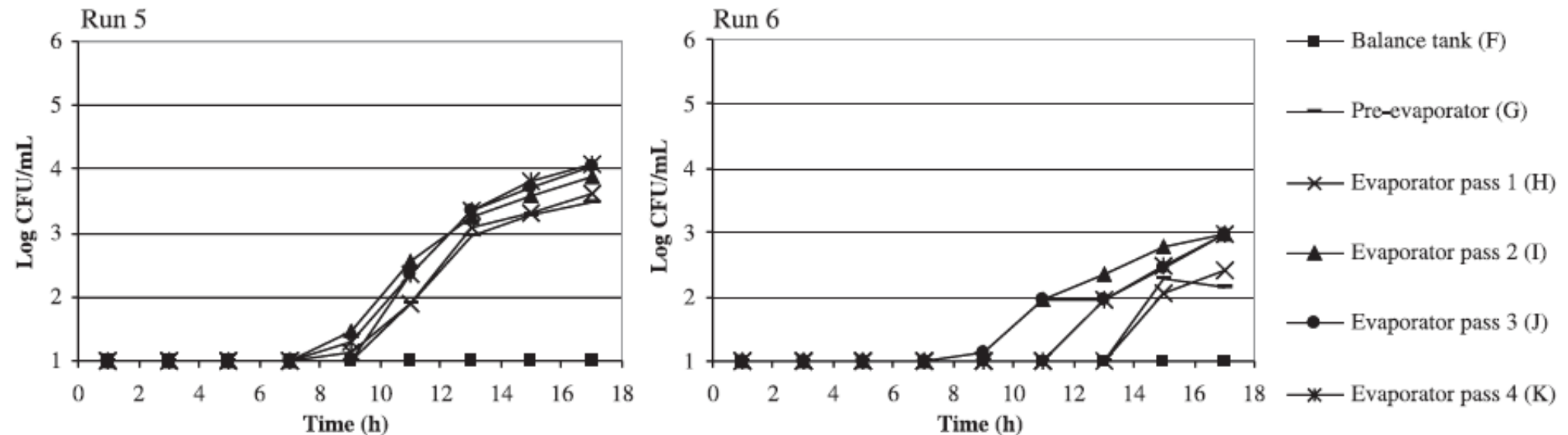
## ***Post-processing contamination***

- Inadequate cleaning and sanitization leading to biofilm formation
- Indicators of PPC in fluid milk include Gram-negative bacteria (e.g., *Pseudomonas*)
- Sporeformers can also be introduced into processed dairy products through biofilm formation



"Biofilm" by D. Davis - From: Looking for Chinks in the Armor of Bacterial Biofilms Monroe D PLoS Biology Vol. 5, No. 11, e307 doi:10.1371/journal.pbio.0050307  
<http://biology.plosjournals.org/perlserv/?request=slideshow&type=figure&doi=10.1371/journal.pbio.0050307&id=89595>. Licensed under CC BY 2.5 via Wikimedia Commons -  
<https://commons.wikimedia.org/wiki/File:Biofilm.jpg#media/File:Biofilm.jpgprocessing>

# Sporeforming bacteria in processing environments



**Figure 4** Thermophilic spore counts of samples taken every 2 h over an 18-h run period of two standard whole milk powder runs (runs 5 and 6). Each point represents the mean of triplicate counts.

# Testing methods matter

---

## Spore Pasteurization

80°C (176°F)/12m

PSC

6°C (42.8°F)/10d  
incubation

MSC

32°C (89.6°F)/48h  
incubation

TSC

55°C (131°F)/48h  
incubation

## Highly Heat Resistant Spore Treatment

100°C (212°F)/30m

HHR-MSC

32°C/48h incubation

HHR-TSC

55°C/48h incubation

## Specially Heat Resistant Spore Treatment

106°C (222.8°F)/30m

STSC

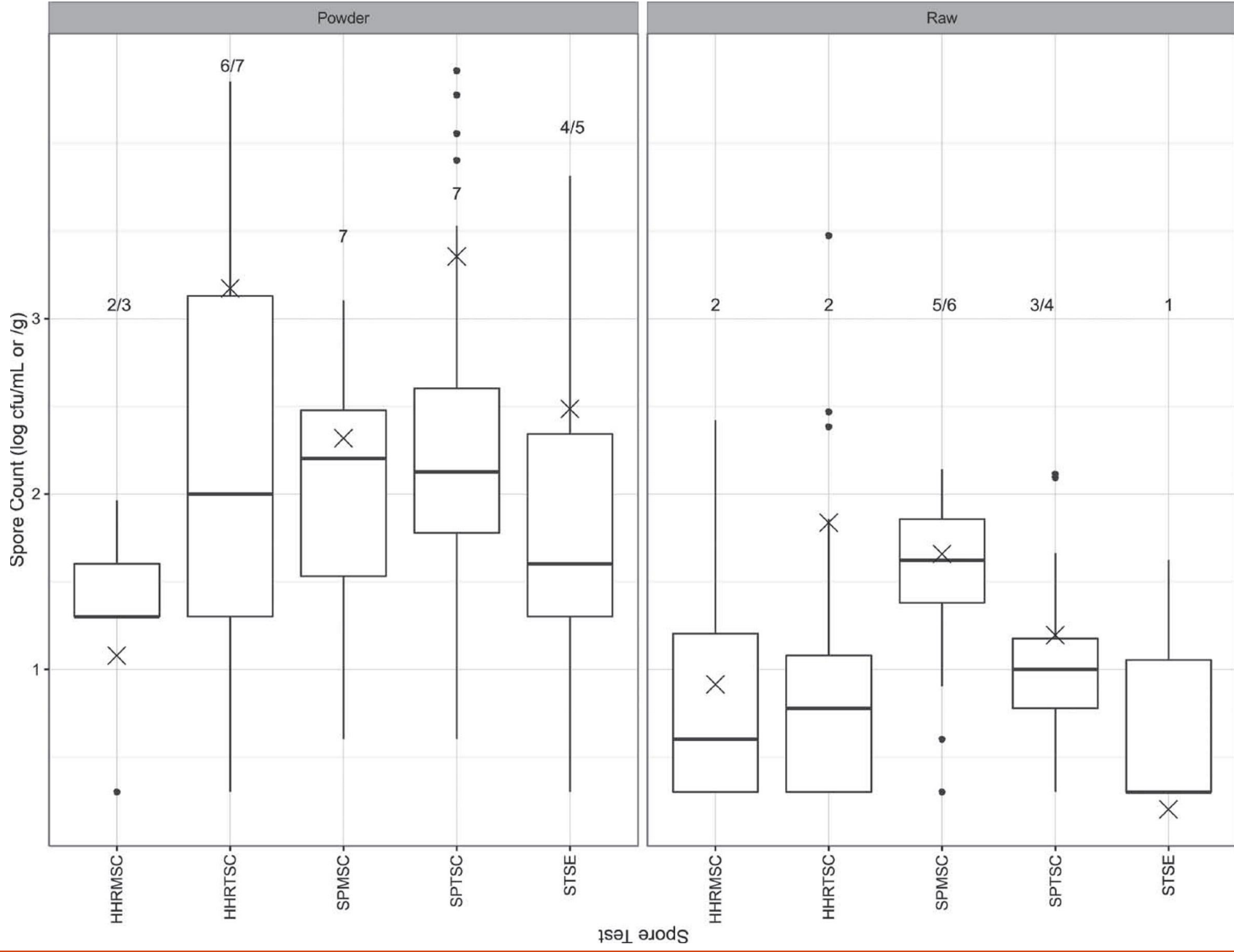
55°C/48h incubation

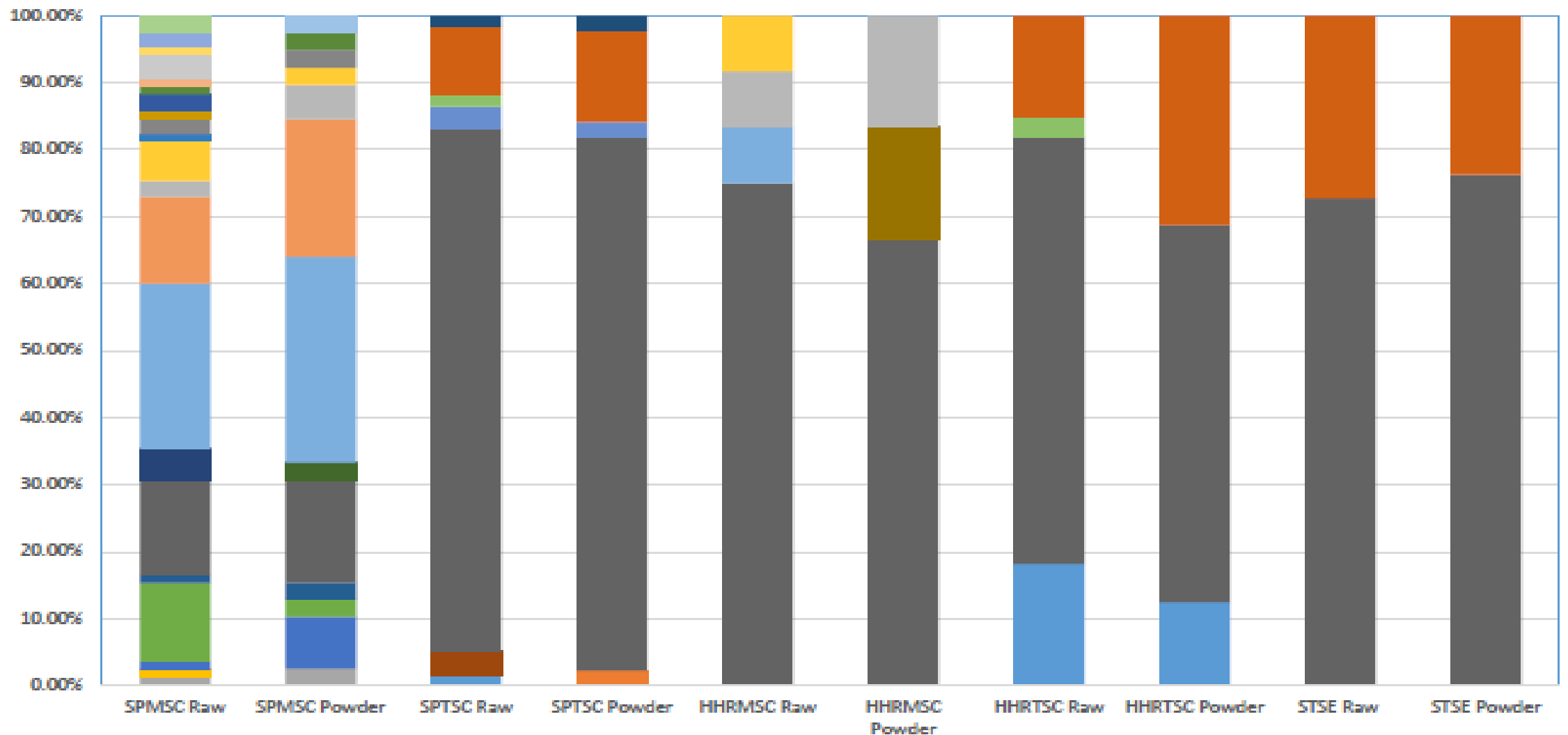
## Butyric Acid Bacteria (BAB)

75°C (167°F)/15m

BAB Most Probable  
Number (MPN)

37°C (98.6°F)/7d  
incubation





- *Aeribacillus pallidus*
- *Bacillus cereus* s.l.
- *Bacillus licheniformis*
- *Bacillus pumilus*
- *Bacillus thermoamylovorans*
- *Lysinibacillus* sp.
- *Paenibacillus cf. illinoisensis*
- *Psychrobacillus cf. psychrotolerans*

- *Anoxybacillus* sp.
- *Bacillus cf. aerophilus*
- *Bacillus megaterium*
- *Bacillus safensis*
- *Brevibacillus borstelensis*
- *Oceanobacillus neutrophilus/sojajae*
- *Paenibacillus cf. sonchii*
- *Sporosarcina cf. globispora*

- *Bacillus altitudinis*
- *Bacillus cf. nealsonii*
- *Bacillus cf. mojavensis*
- *Bacillus sp.*
- *Brevibacillus cf. parabrevis*
- *Oceanobacillus* sp.
- *Paenibacillus odorifer*
- *Ureibacillus thermosphaericus*

- *Bacillus atrophaeus*
- *Bacillus coagulans*
- *Bacillus oleronius*
- *Bacillus subtilis* s.l.
- *Geobacillus* sp.
- *Paenibacillus amylolyticus* s.l.
- *Paenibacillus* sp.

# Spore populations in raw milk vs. dairy powders

**Table 3.** Spore genera and species isolated from samples from 4 different dairy powder processing plants and bulk tank raw milk from 33 farms

Genus and species	Spore isolates from each source, <sup>1</sup> % (no. of isolates)					
	SW	NDM	AW	WPC-80	BT raw milk	Total
<i>Aeribacillus pallidus</i>	0.6 (2)	2.1 (8)	0.0 (0)	4.3 (8)	1.3 (8)	1.7 (26)
<i>Anoxybacillus</i> spp.	38.0 (126)	9.4 (35)	0.0 (0)	0.0 (0)	0.0 (0)	10.6 (161)
<i>Bacillus cereus</i> group	17.2 (57)	1.3 (5)	0.0 (0)	13.3 (25)	4.7 (28)	7.6 (115)
<i>Bacillus clausii</i>	0.3 (1)	2.1 (8)	5.9 (2)	0.5 (1)	5.2 (31)	2.8 (43)
<i>Bacillus licheniformis</i>	6.3 (21)	44.9 (168)	73.5 (25)	31.9 (60)	47.4 (282)	36.5 (556)
<i>Bacillus pumilus</i>	0.0 (0)	6.4 (24)	0.0 (0)	0.5 (1)	9.1 (54)	5.2 (79)
<i>Bacillus subtilis</i> s.l.	2.4 (8)	1.1 (4)	0.0 (0)	2.7 (5)	4.9 (29)	3.0 (46)
<i>Bacillus thermoamylovorans</i>	0.0 (0)	2.1 (8)	2.9 (1)	16.0 (30)	6.4 (38)	5.1 (77)
<i>Geobacillus</i> spp.	27.1 (90)	16.0 (60)	0.0 (0)	18.1 (34)	0.2 (1)	12.1 (185)
<i>Lysinibacillus</i> spp.	0.0 (0)	1.3 (5)	0.0 (0)	1.1 (2)	3.2 (19)	1.7 (26)
<i>Paenibacillus</i> spp.	0.9 (3)	3.5 (13)	2.9 (1)	0.5 (1)	4.2 (25)	2.8 (43)
<i>Ureibacillus</i> spp.	0.3 (1)	1.1 (4)	0.0 (0)	1.6 (3)	0.3 (2)	0.7 (10)
Other <sup>2</sup>	0.9 (3)	1.1 (4)	5.9 (2)	0.0 (0)	2.4 (14)	1.5 (23)
Other <i>Bacillus</i> spp. <sup>3</sup>	6.0 (20)	7.5 (28)	8.8 (3)	9.6 (18)	10.8 (64)	8.7 (133)
Total isolates, n	332	374	34	188	595	1,523

<sup>1</sup>SW = sweet whey; NDM = nonfat dry milk; AW = acid whey; WPC-80 = whey protein concentrate 80; BT = bulk tank.

<sup>2</sup>Other includes *Brevibacillus* spp., *Oceanobacillus* spp., *Solibacillus* spp., *Sporosarcina* spp., and *Terribacillus* spp.

<sup>3</sup>Other *Bacillus* spp. includes species *aerophilus*, *coagulans*, *firmus*, *gibsonii*, *horneckiae*, *megaterium*, *muralis*, *nealsonii*, *neutrophilus/sojae*, *safensis*, *smithii*, and other *Bacillus* spp.

# Spore testing challenges

---

- Low level contamination, stochastic variation
- Population diversity
- Germination and recovery variability on different media
- “Countability”
  - Swarmers, spreaders and microaerophiles, oh my!

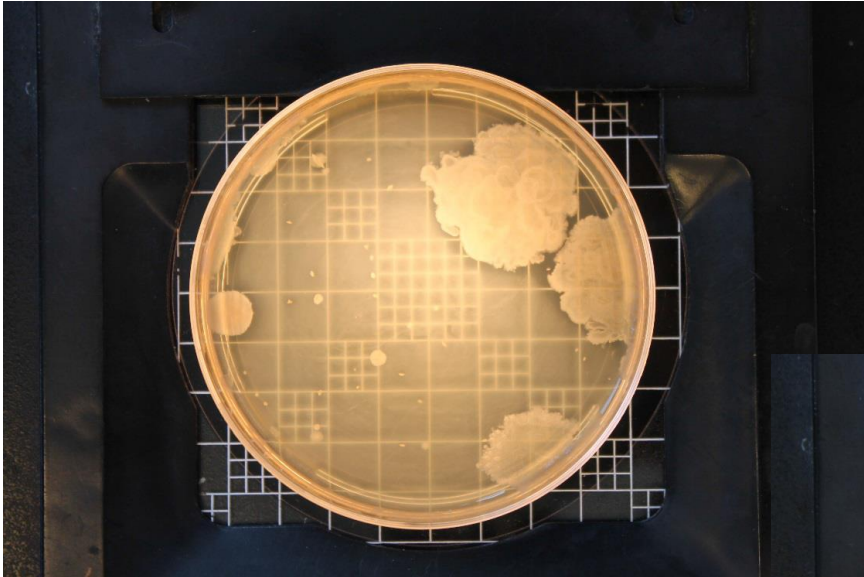
*What is the “right” method?  
Must consider implementation*

# Spore enumeration

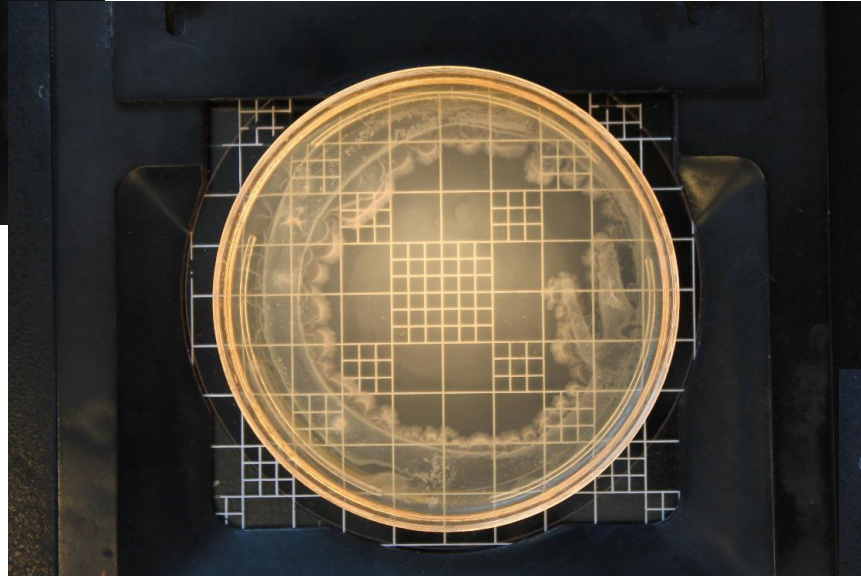
---



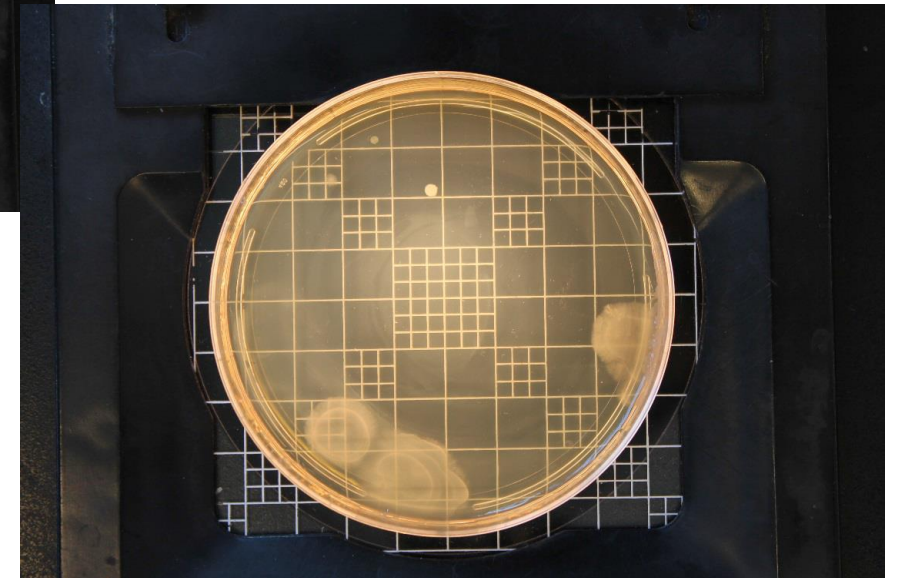
Milk powder A, SP TSC on BHI, Pour Plate



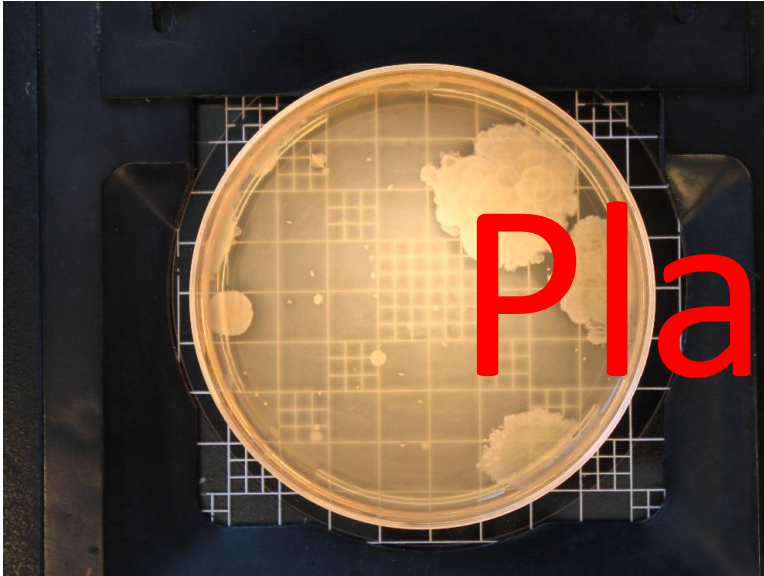
Milk powder A, SP TSC on BHI, Spread plate



Milk powder A, SP TSC on BHI, Pour Plate with overlay



Milk powder A, SP TSC on BHI, Pour Plate

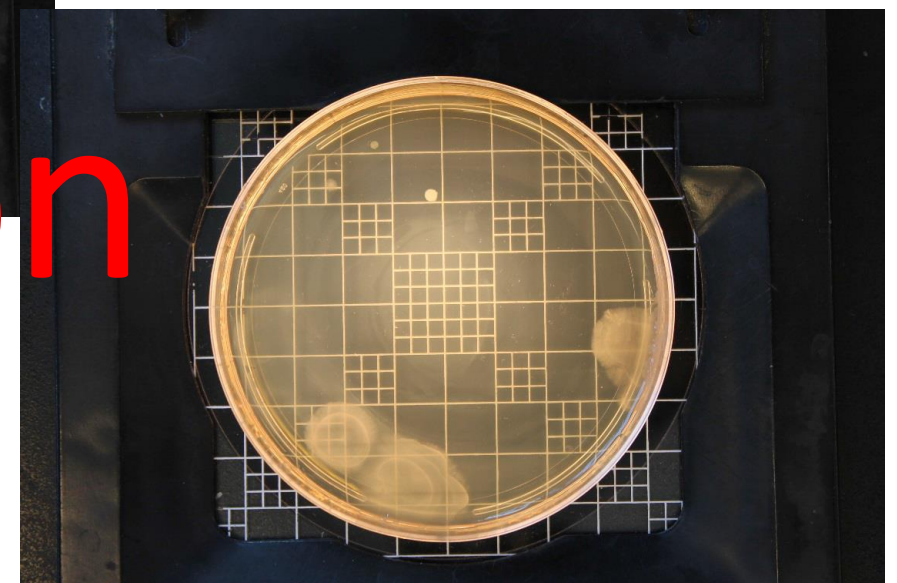


Plating type is a

source of

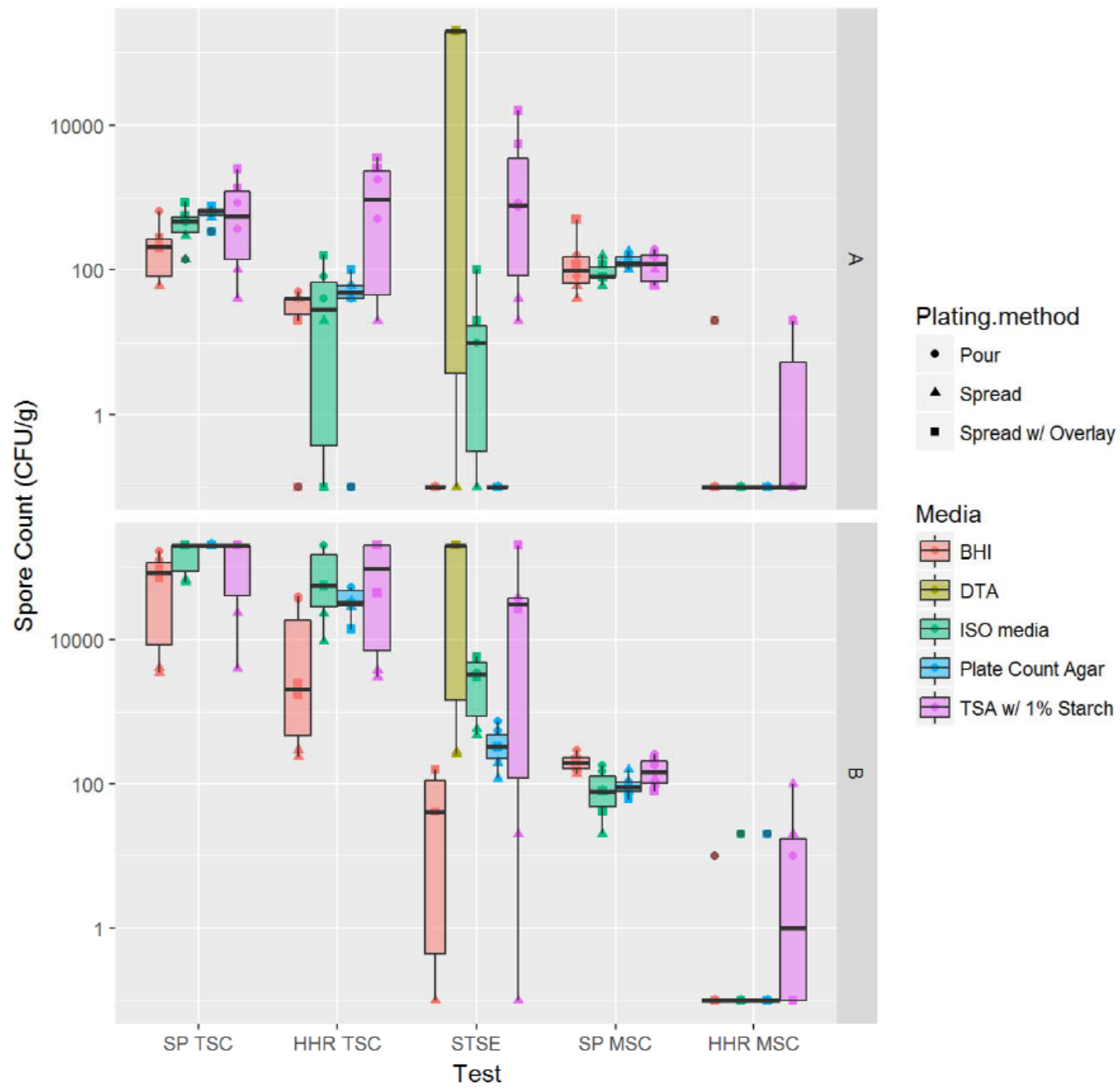
variation

Milk powder A, SP TSC on BHI, Spread plate



Milk powder A, SP TSC on BHI, Pour Plate with overlay

Count by Media within Test



# Summary and moving forward

---

- Spores are ubiquitous and must be controlled at the farm and processing level
- Sample type (raw milk, powder, etc), Media type, plating type, heat treatment and incubation parameters all play an important role in spore method variation
  - Populations in different dairy products can vary widely contributing to variation seen between spore testing methods
- Identify a set of methods and parameters that provide standards for spore testing

# Acknowledgments

---

- NYFVI
- NYSDPAB
- NDC
- Staff and students of the Milk Quality Improvement Program and Food Safety Laboratory
- Participating producers, cooperatives and processors