

Indicator organisms and their uses in the dairy industry

NICOLE MARTIN, MS

ASSOCIATE DIRECTOR, MILK QUALITY IMPROVEMENT PROGRAM

CORNELL UNIVERSITY



Indicators vs. Index Organisms

- Indicator organism
 - Markers whose presence relates to the general microbiological condition of the food or environment (i.e., hygienic quality)
- Index organisms
 - Markers whose presence relates to the possible occurrence of ecologically similar pathogens

Indicator organisms cannot be used as index organisms

Indicator organisms: A long history of use in the food and water industry

- Coliforms have been used since the late 19th century as an indicator of fecal contamination in drinking water
- The US dairy industry adopted coliforms as indicators of unsanitary processing conditions as early as 1924
- Methodology for detecting coliforms has evolved since its inception, recent advances in taxonomy have led to new insights into the coliform group and its usefulness as an indicator organism

Frequently used dairy foods indicator organisms

■ Coliforms

- Hygiene and sanitation indicator
- Aerobic and facultatively anaerobic, Gram-negative, non-sporeforming rods capable of fermenting lactose to produce gas and acid within 48h at 32-37C
- Composed of 19 genera primarily within the Enterobacteriaceae family – Method defined
- In the US coliform testing is required by the PMO

Frequently used dairy foods indicator organisms, cont.

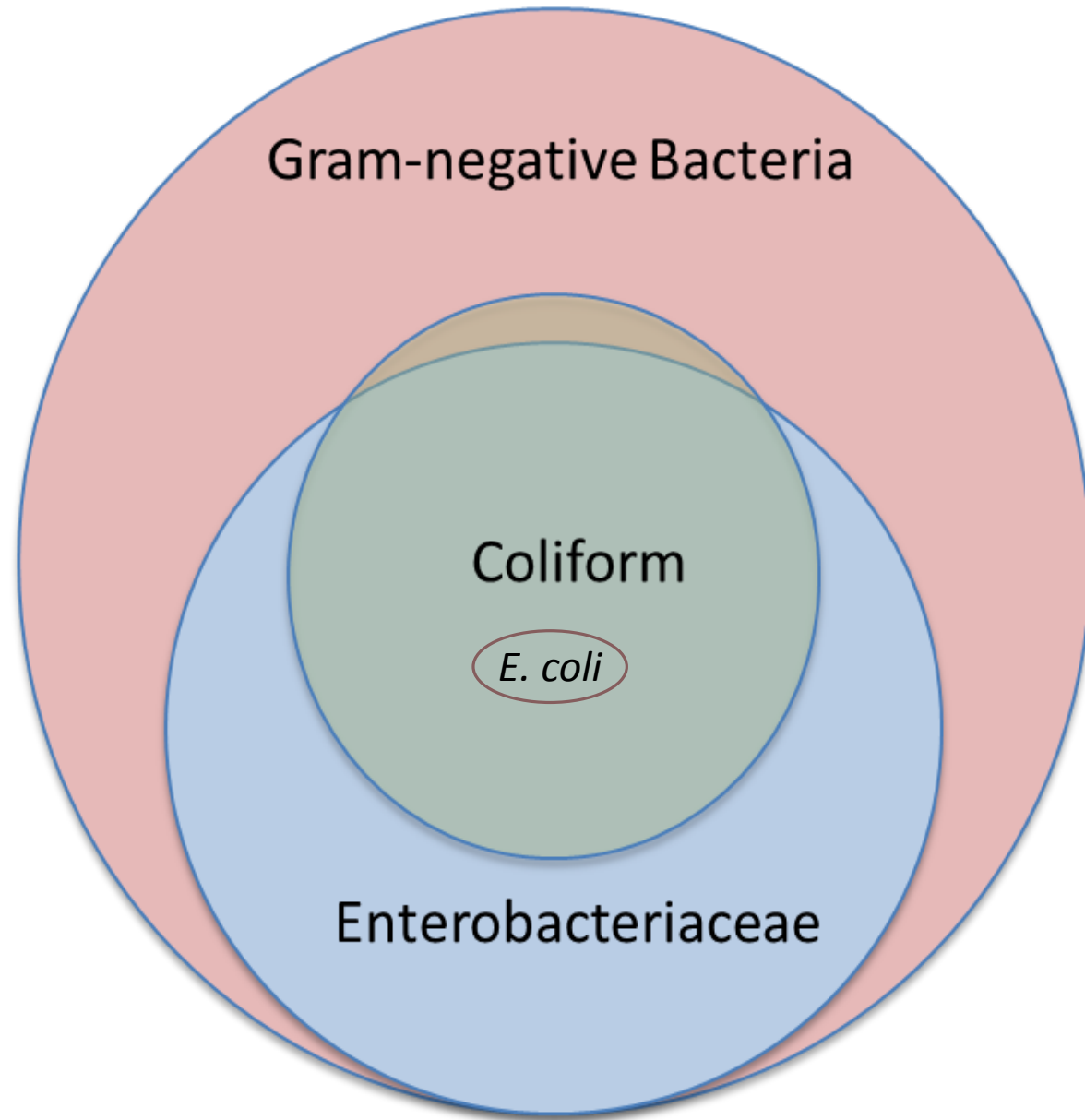
- Enterobacteriaceae
 - Hygiene and sanitation indicator
 - Facultatively anaerobic, Gram-negative, non-sporeforming rods capable of fermenting glucose to produce gas and acid within 48h at 32-37C
 - Primary indicator organisms used in Europe

Frequently used dairy foods indicator organisms, cont.

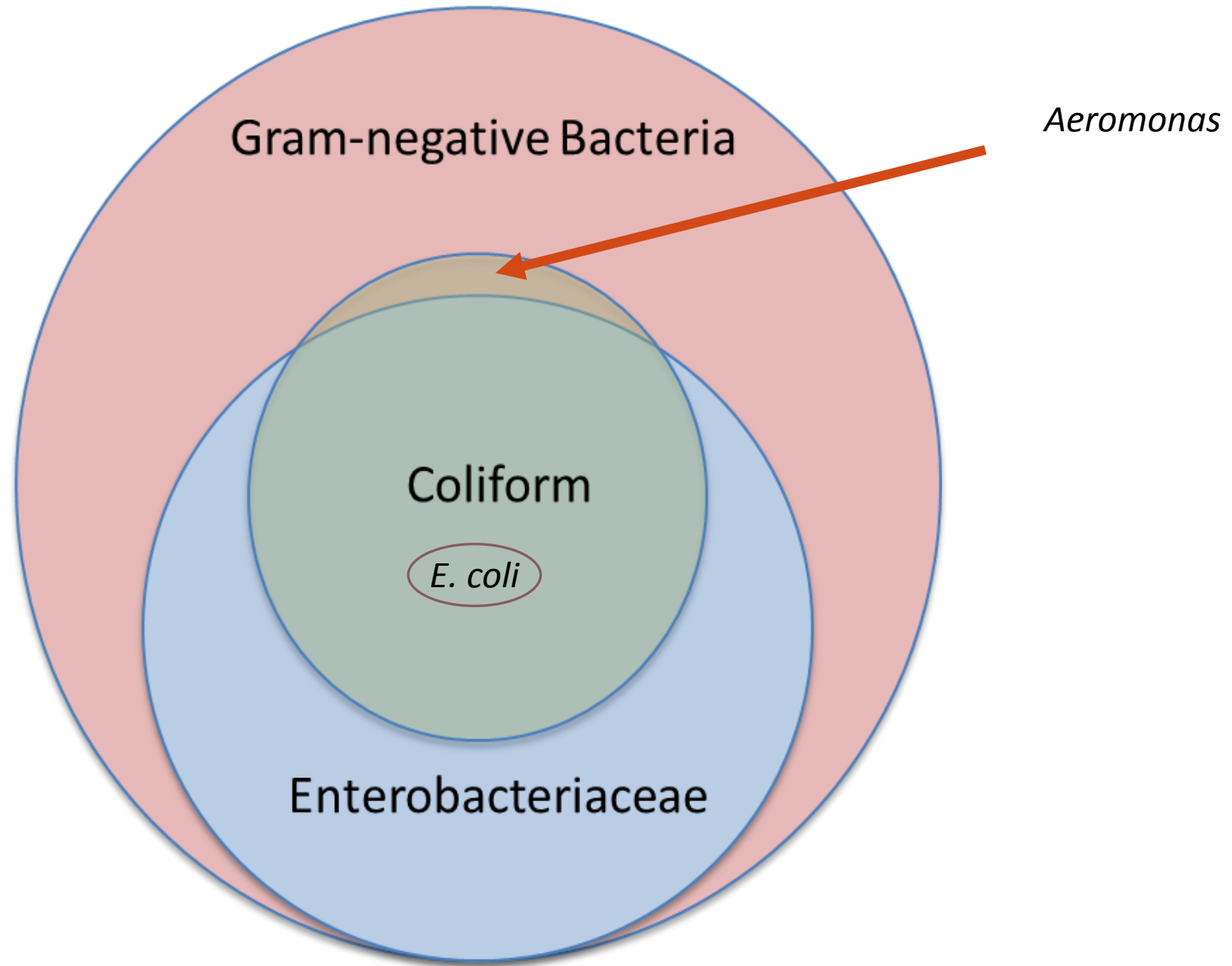
- Total Gram-negatives
 - Hygiene and sanitation indicator
 - All Gram-negative bacteria are eliminated by pasteurization, meaning that their presence in a processed dairy product is an indication of contamination
 - Current methods rely on traditional culture techniques

Frequently used dairy foods indicator organisms, cont.

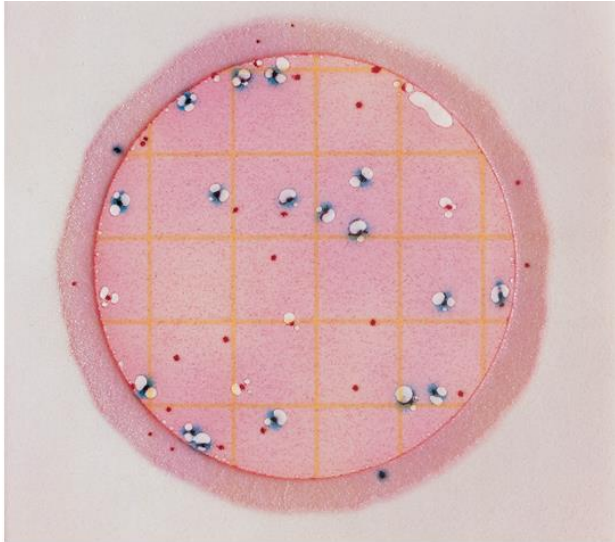
- *Escherichia coli*
 - Thermotolerant coliform - grows and ferments lactose at 44-45°C
 - The only “member” of the coliform group that can be used as an indicator of fecal contamination (some strains of *E. coli* can also be environmental contaminants)



Gram-negative bacteria and subsets therein are used as indicators of process failure and post-processing contamination because they are eliminated by pasteurization

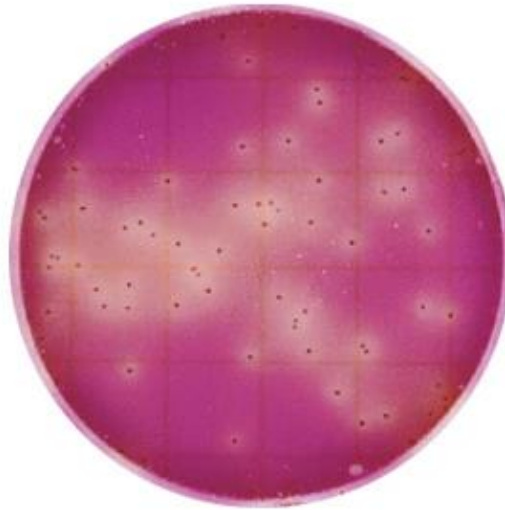


Indicator organisms: Culture methods



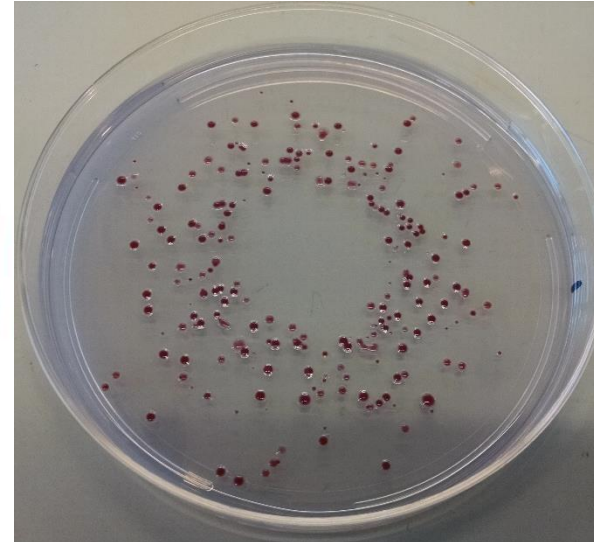
Coliform Count (CC) Petrifilm

- Rapid (24 h)
- Convenient
- Detects only coliforms
- Required by PMO



Enterobacteriaceae (EB) Petrifilm

- Rapid (24 h)
- Convenient
- Detects only Enterobacteriaceae



Crystal Violet Tetrazolium Agar (CVTA)

- Less rapid (48 h)
- Less convenient
- Detects nearly all Gram-negative PPC

Indicator organisms: Rapid methods



Frequently used dairy foods indicator organisms, cont.

- Enterococci
 - Hygiene and sanitation indicator in fermented dairy products
 - Gram positive cocci, facultative anaerobic, tolerant of a wide range of environmental conditions
 - Very little research to support the use of Enterococci in cultured dairy products

Frequently used dairy foods indicator organisms, cont.

- Yeast and Mold

- Useful in cultured products where fungal organisms survive better than bacteria
- Typically much slower total testing time

- Aerobic Plate Count

- Useful in commercially sterile products

Goal of using indicator organisms in dairy products

*Quickly identify lapses in cleaning/sanitation, GMPs, PM, etc. in order to resolve the issue and prevent further compromises in **quality***

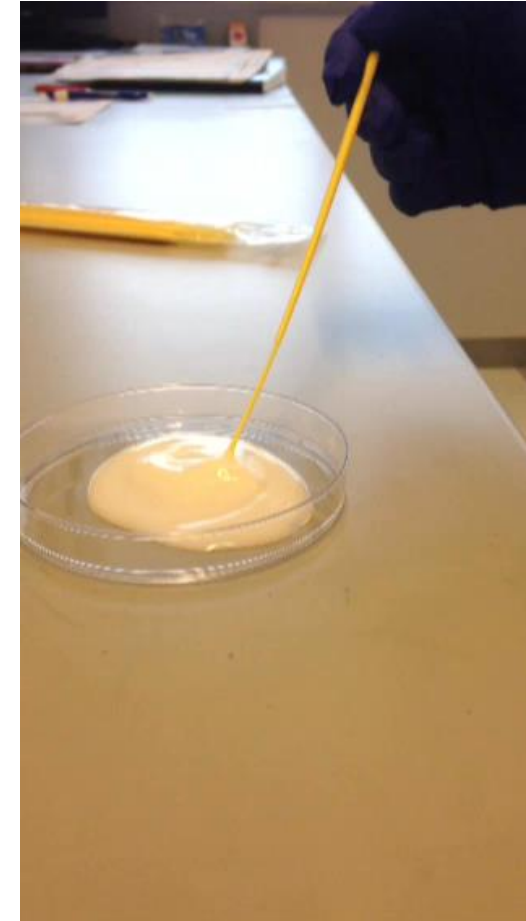
Gray milk

(Pseudomonas)



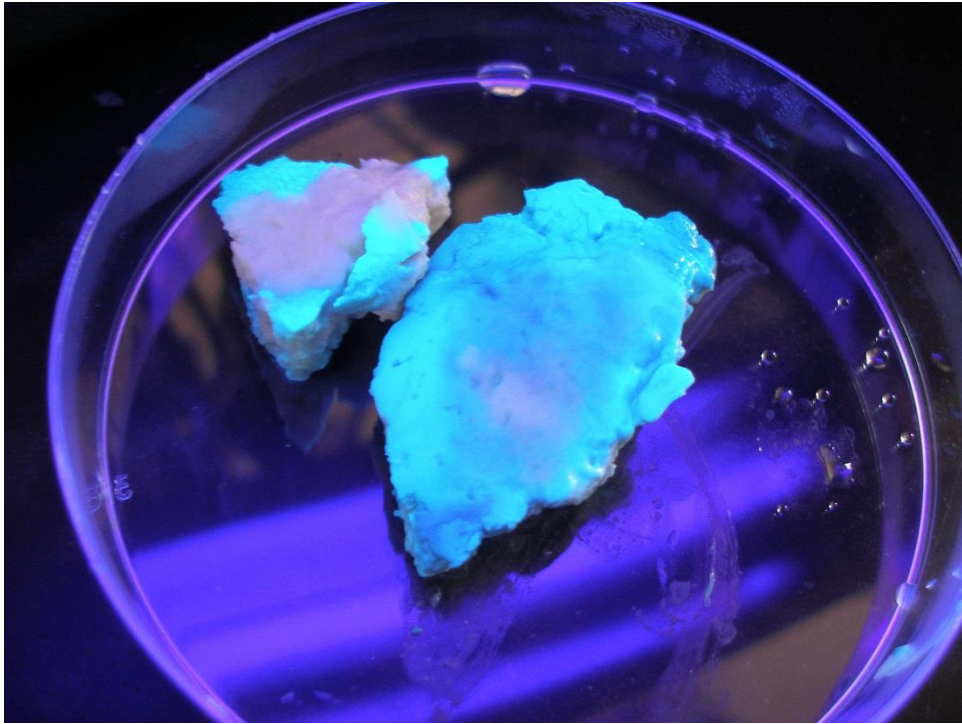
Ropy milk

(Rahnella, Klebsiella)



Blue Cheese

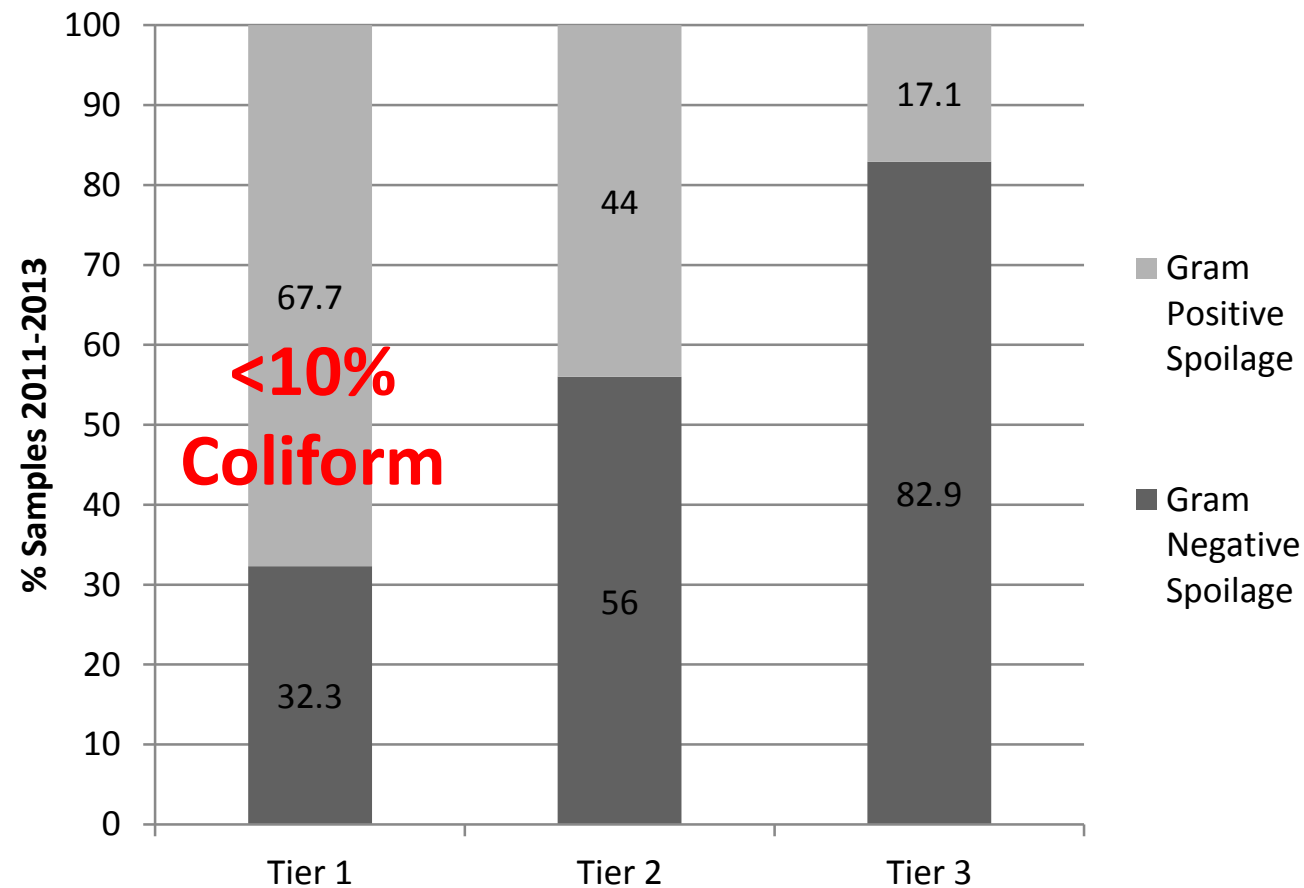
(Pseudomonas)



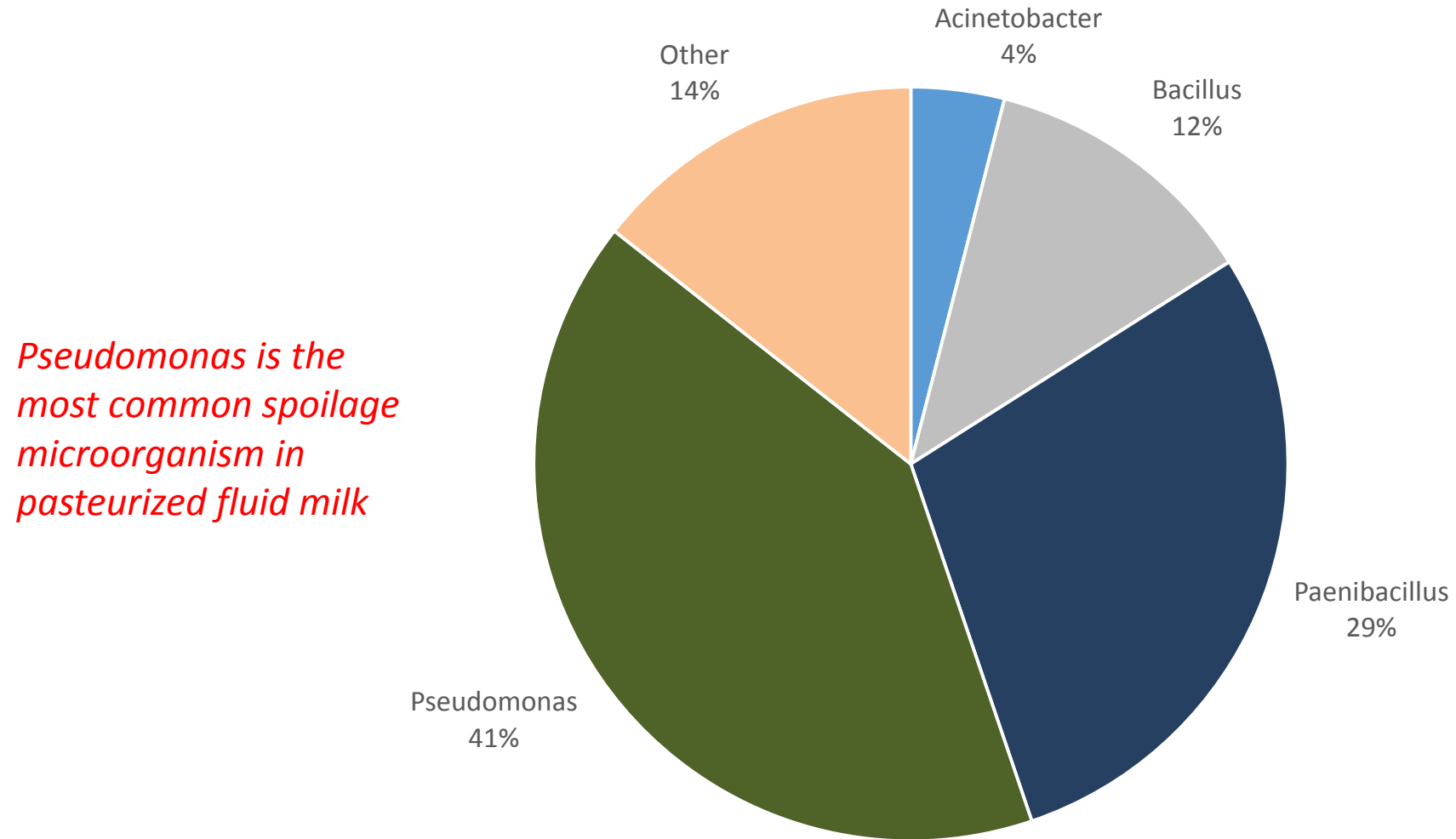
Hallmarks of an appropriate indicator organisms

- Survives in product
- Rapid
- Easy to test
- Accurate

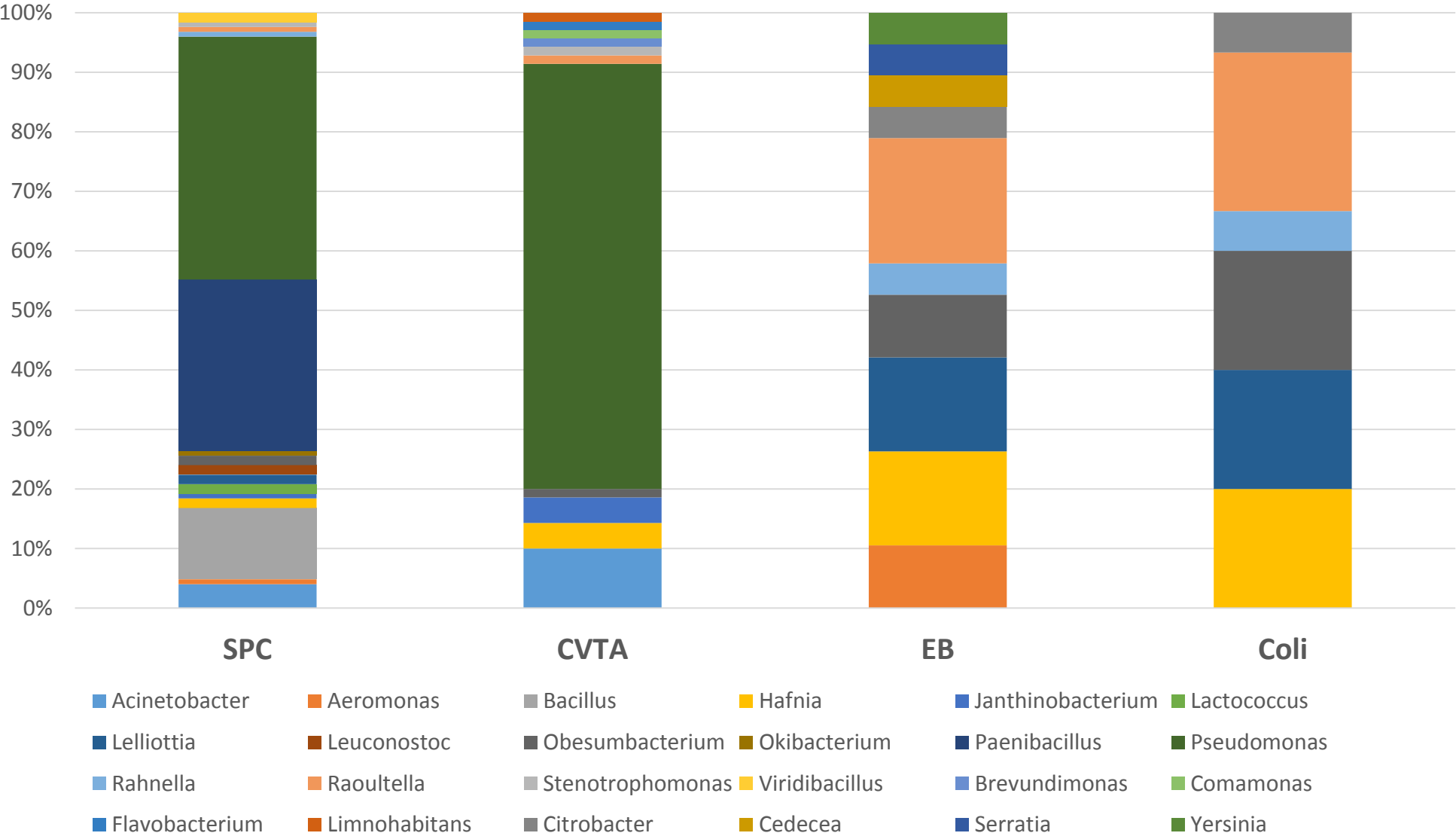
Using appropriate indicator organisms: A fluid milk case study



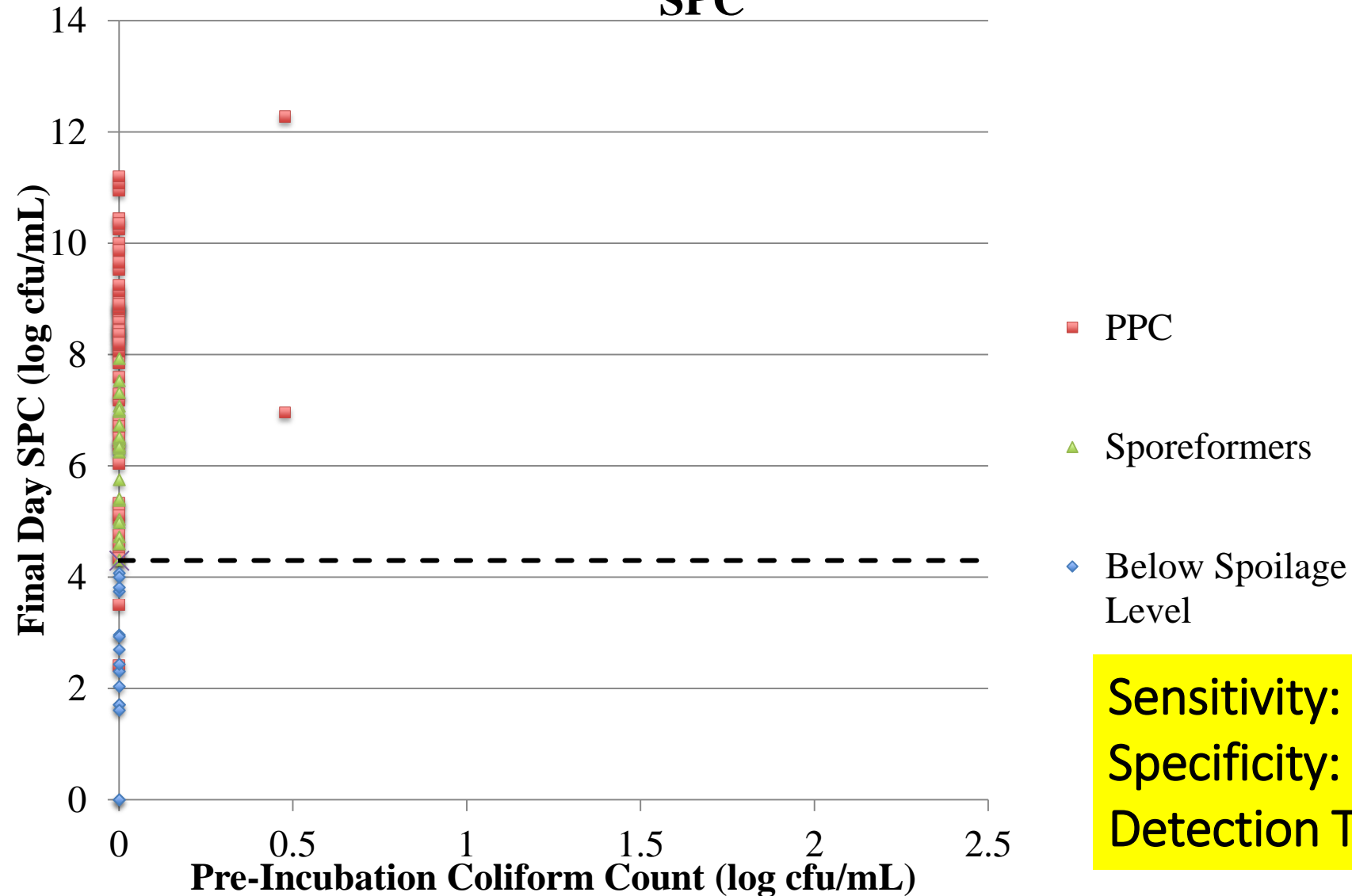
Microbial Ecology of Pasteurized Fluid Milk



Microbial Ecology of Pasteurized Fluid Milk

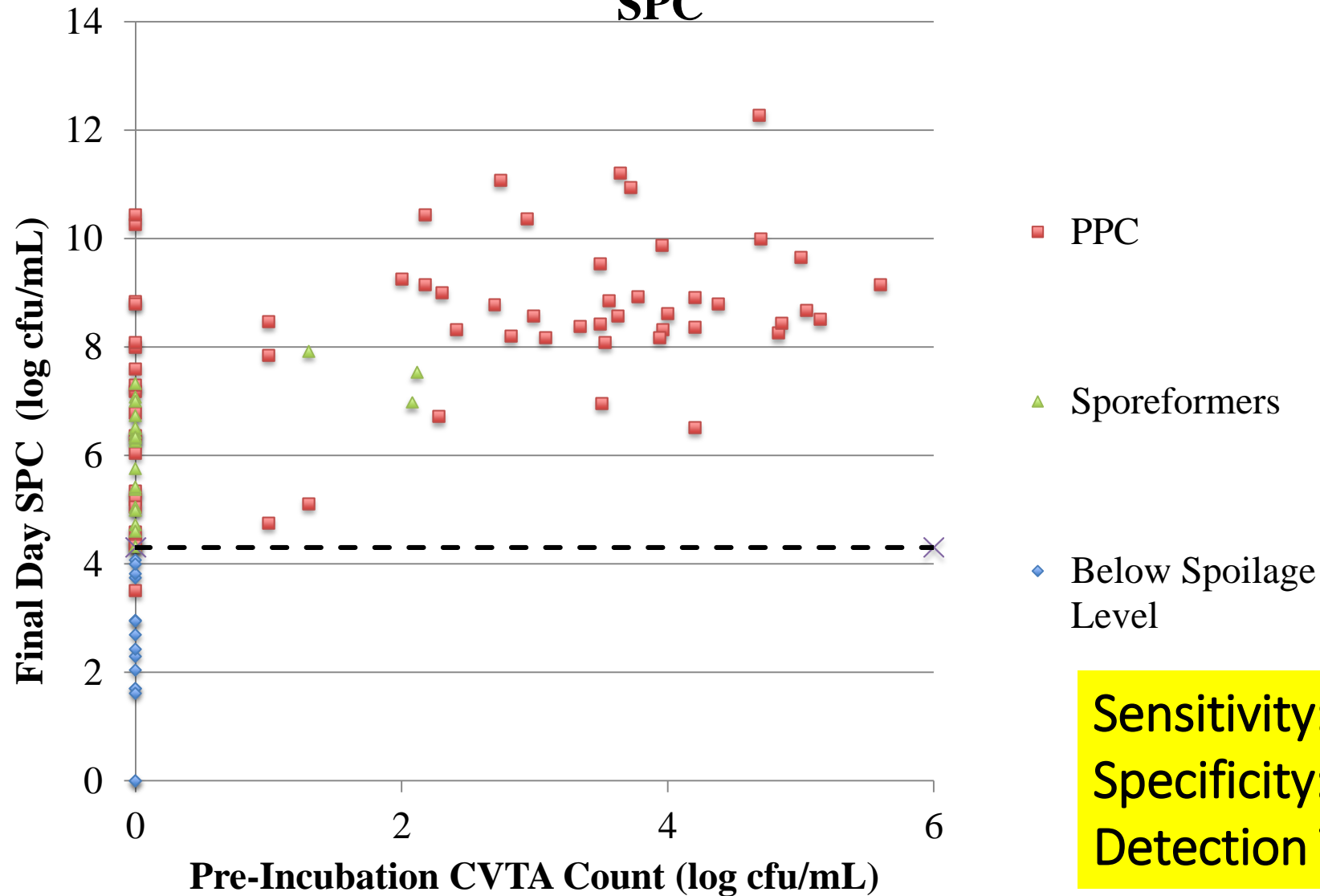


Pre-Incubation Coliform Counts (13°C) vs. Final Day SPC



Sensitivity: 20%
Specificity: 99%
Detection Time: ≤30h

Pre-Incubation CVTA Counts (21°C) vs. Final Day SPC

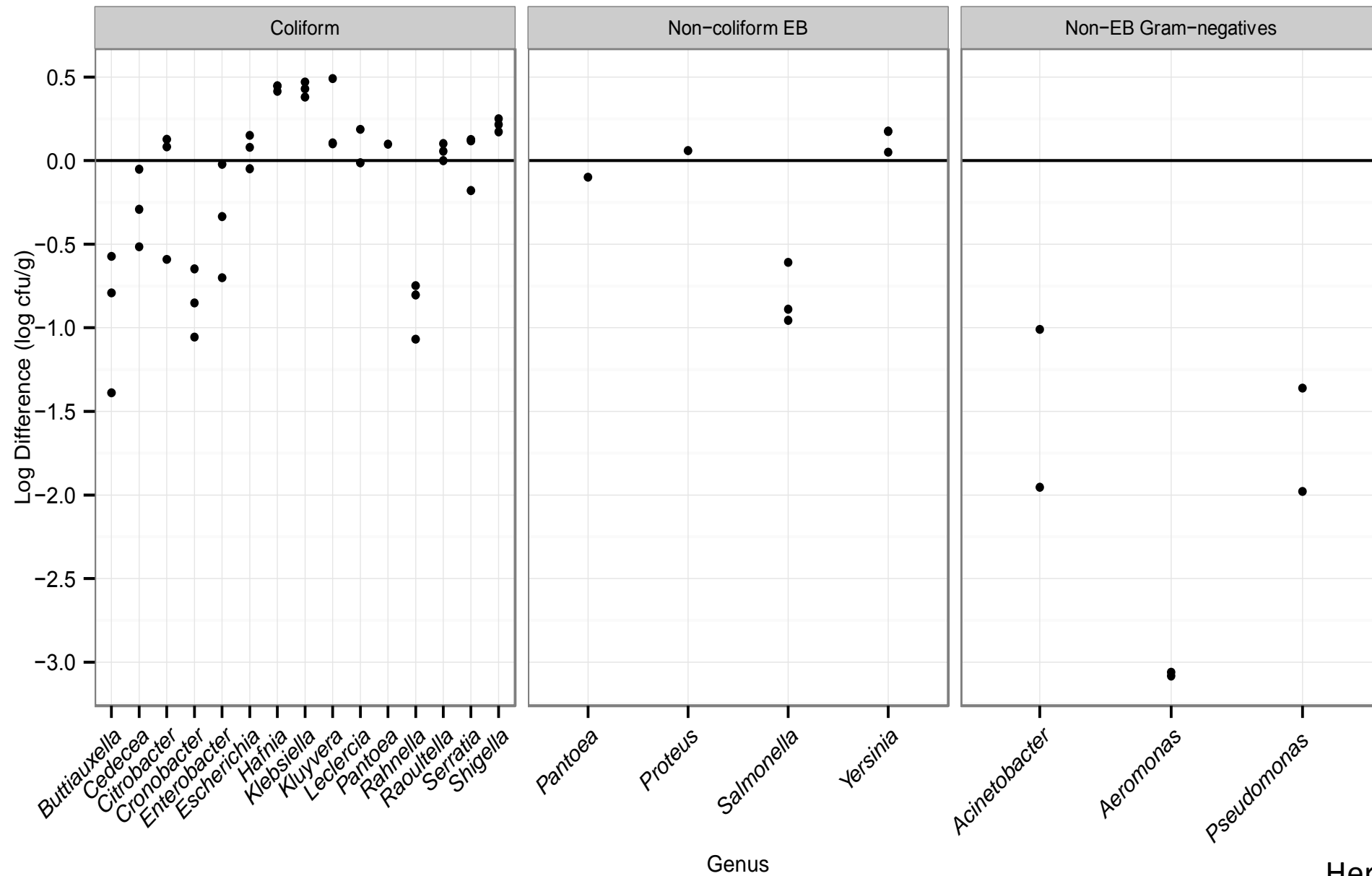


Comparing indicator organisms: Fluid milk

Indicator Organism	Survives in Product?	Rapid?	Easy to Test?	Accurate?
Coliforms	✓	✓	✓	✗
Enterobacteriaceae	✓	✓	✓	✗
Total Gram-Negative Bacteria	✓	✓ *	✓ *	✓

Using appropriate indicator organisms: A greek yogurt case study

- Coliforms and EB are currently used as indicator organisms in yogurt products
- Many spoilage bacteria do not survive* in the low pH yogurt environment – a challenge for testing
- Enterococci have been suggested as an alternative to coliform and EB testing
 - Survive in high acid environments, but are infrequent contaminants in yogurt products



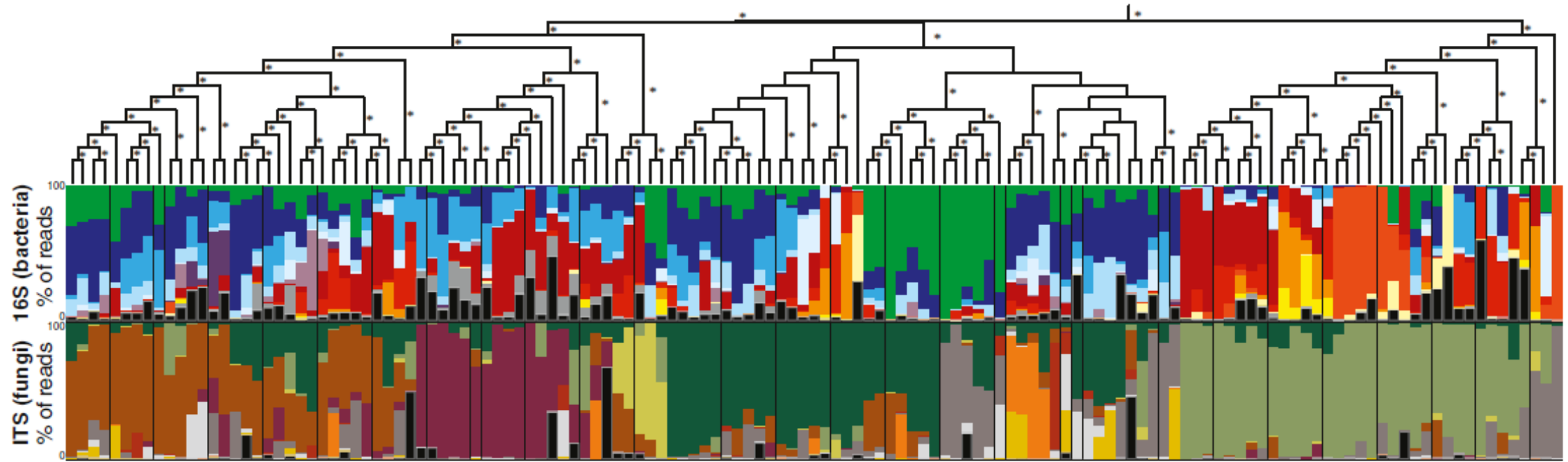
Hervert et al., 2016

Comparing indicator organisms: Yogurt

Indicator Organism	Survives in Product?	Rapid?	Easy to Test?	Accurate?
Coliforms	✓ *	✓	✓	✓
Enterobacteriaceae	✓ *	✓	✓	✓
Total Gram-Negative Bacteria	✗	✓ *	✓ *	✗
Enterococci	✓	✓ *	✓ *	✗

Using appropriate indicator organisms: A cheese case study

- Coliforms and EB are currently used as indicator organisms in cheese products and have regulatory limits
- In some cheese products (i.e., natural rind cheese), coliforms are a beneficial part of the microflora making their use as indicator organisms challenging
 - Cultures known to be in the coliform group have also been developed for use in cheese applications
- The diversity in types of cheese and their characteristics (e.g., pH, a_w , etc) determines the ability of coliforms and EB to survive



Bacteria

- Firmicutes
 - *Staphylococcus*
 - *Brevibacterium*
 - *Corynebacterium*
- Actinobacteria
 - *Brachybacterium*
 - *Arthrobacter*
 - *Nocardiopsis*
 - *Yaniella*

- Proteobacteria
 - *Halomonas*
 - *Psychrobacter*
 - *Pseudomonas*
 - *Pseudoalteromonas*
 - *Vibrio*
 - *Hafnia/Serratia*
- Bacteroidetes
 - *Sphingobacterium*
- Taxa < 1% ave. abund.

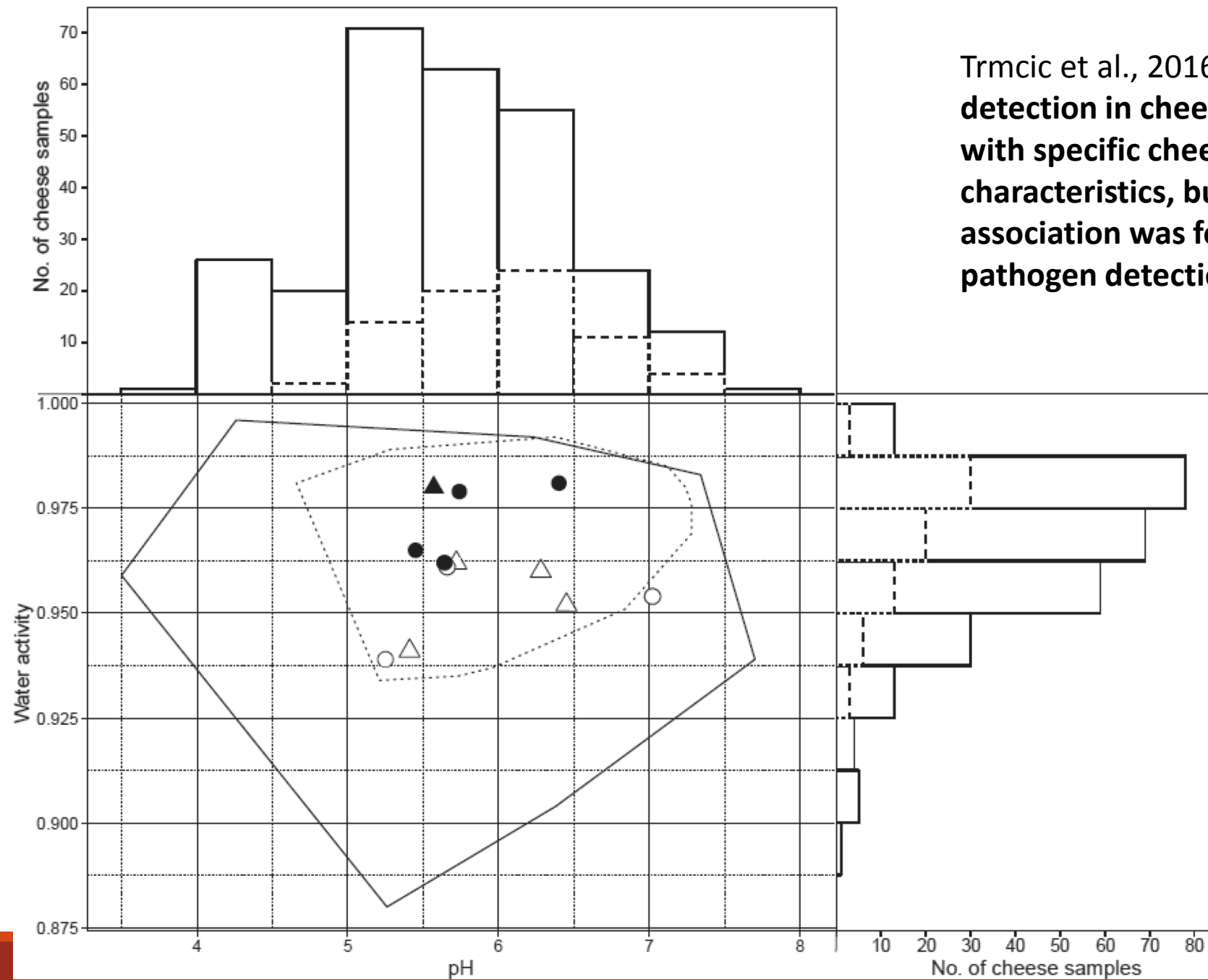
Fungi

- Saccharomycetales
 - *Debaryomyces*
 - *Galactomyces*
 - *Candida*
- Microascales
 - *Scopulariopsis*
- Hypocreales
 - *Fusarium*
 - *Acremonium*

- Eurotiales
 - *Penicillium*
 - *Aspergillus*
- Onygenales
 - *Sporendonema*
 - *Chrysosporium*
- Taxa < 1% ave. abund.

Finding appropriate indicator organisms for cheese products

- In certain cheese products, EB or *E. coli* may be appropriate indicators
 - Processed cheese products, fresh cheese, etc.
 - More research needed
- Aged cheese products represent more of a challenge when identifying appropriate indicator organisms
 - There is no association between the presence of coliforms and relevant pathogens (i.e., *Listeria monocytogenes*)
 - Current research indicates that targeted risk-based pathogen testing in aged cheese based on cheese characteristics



Trmcic et al., 2016 **Coliform detection in cheese is associated with specific cheese characteristics, but no association was found with pathogen detection**

*Rethinking indicator organisms –
a way forward*

TABLE 2 | Proposed hygiene indicator tests for different dairy products.

Product	Proposed microbial hygiene indicator test ²	Justification	Key references
Fluid milk	Total Gram-negative bacteria	Key hygienic issues in pasteurized fluid milk are (i) PPC and (ii) pasteurization failure. Both can be detected more reliably with a test that detects all GN bacteria (rather than coliform or Enterobacteriaceae [EB] tests).	Ranieri and Boor, 2009; Martin et al., 2012
Fermented dairy products (e.g., yogurt, kefir, etc)	Enterobacteriaceae (EB)	Non-EB Gram-negative bacteria decline rapidly at the pH encountered in fermented dairy products while EB generally survive in these conditions making it possible to detect them as indicators of unhygienic conditions.	Hervet, 2016; Hervet et al., 2016
Aged cheeses	Targeted risk-based pathogen testing ¹	No suitable tests are currently available, specific pathogen tests are recommended based on risks associated with specific cheese characteristics (e.g., pH, a_w , etc).	Schwartzman et al., 2014; Trmčić et al., 2016
Fresh cheeses	EB and/or <i>Escherichia coli</i> (additional research needed) ²	Currently coliforms and EB are commonly used as hygienic indicators in fresh cheeses.	
Dairy powders	EB and/or targeted risk-based pathogen testing (additional research needed) ²	Currently coliforms and EB are commonly used as hygienic indicators, but testing for selected pathogens is typically required for dairy powders that are used in infant formula.	
Ice cream	Total Gram-negative bacteria (additional research needed) ²	Currently coliforms and EB are commonly used as hygienic indicators in ice cream.	
Butter	Total Gram-negative bacteria (additional research needed) ²	Currently coliforms, EB, and proteolytic bacteria are commonly used as hygienic indicators.	

¹ Testing for target pathogens of concern may be appropriate for all products (or required under some jurisdictions), even if not specifically mentioned in this Table.

² Proposed indicator tests for these four products (fresh cheese, dairy powders, ice cream, butter) are based on product characteristics, processing parameters and research findings from other dairy products; additional research is needed for these specific products to make more definitive recommendations regarding best practices for microbial hygiene indicator tests.

Challenges to indicator organism testing in dairy products

- Contamination often occurs sporadically and at low levels
 - May require selective enrichment/amplification in order to detect contamination
- Research on appropriate indicator organisms in various dairy products is lacking
 - Coliforms and EB are often used when no data exists to inform decisions

Summary

- Indicator organisms are markers whose presence relates to the general microbiological condition of the food or environment (i.e., hygienic quality) and should not be confused with index organisms
 - Index organisms relate the presence of ecologically similar pathogens (e.g., *Listeria* spp. is an index organism for *Listeria monocytogenes*)
- Appropriately selected indicator organisms are meaningful and allow processors to rapidly respond to lapses in GMPs or process failures
- Coliforms, although by far the indicator organisms with the longest history in the dairy industry are not the best choice in many products (e.g., fluid milk)

Questions?