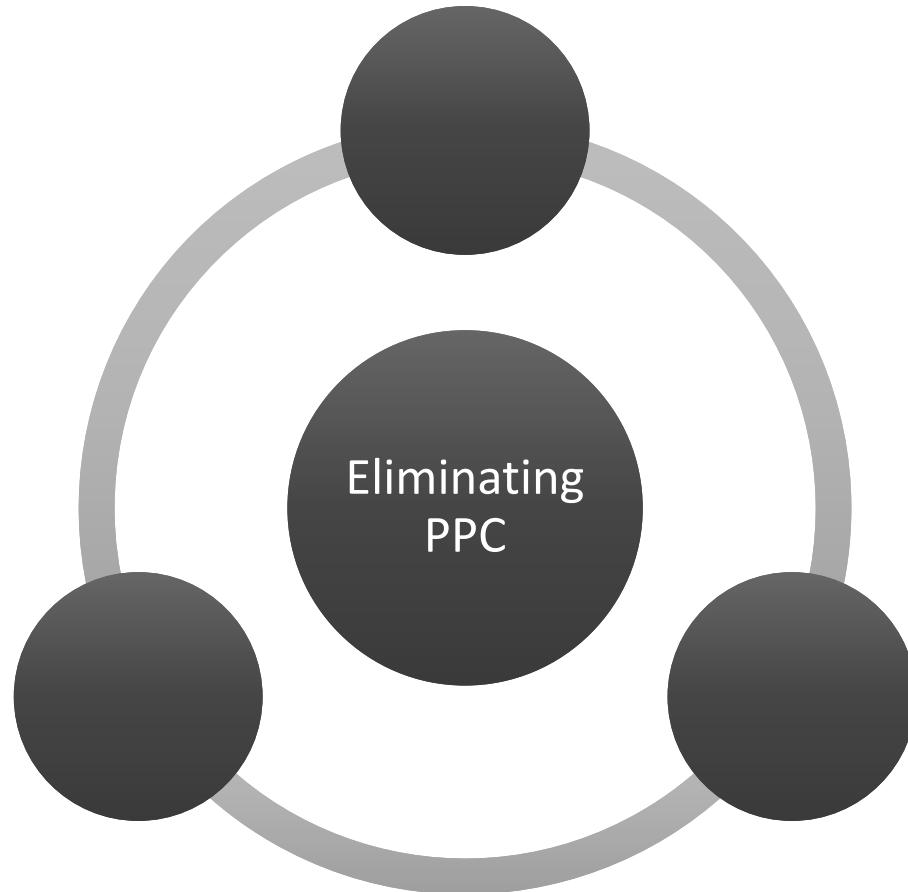
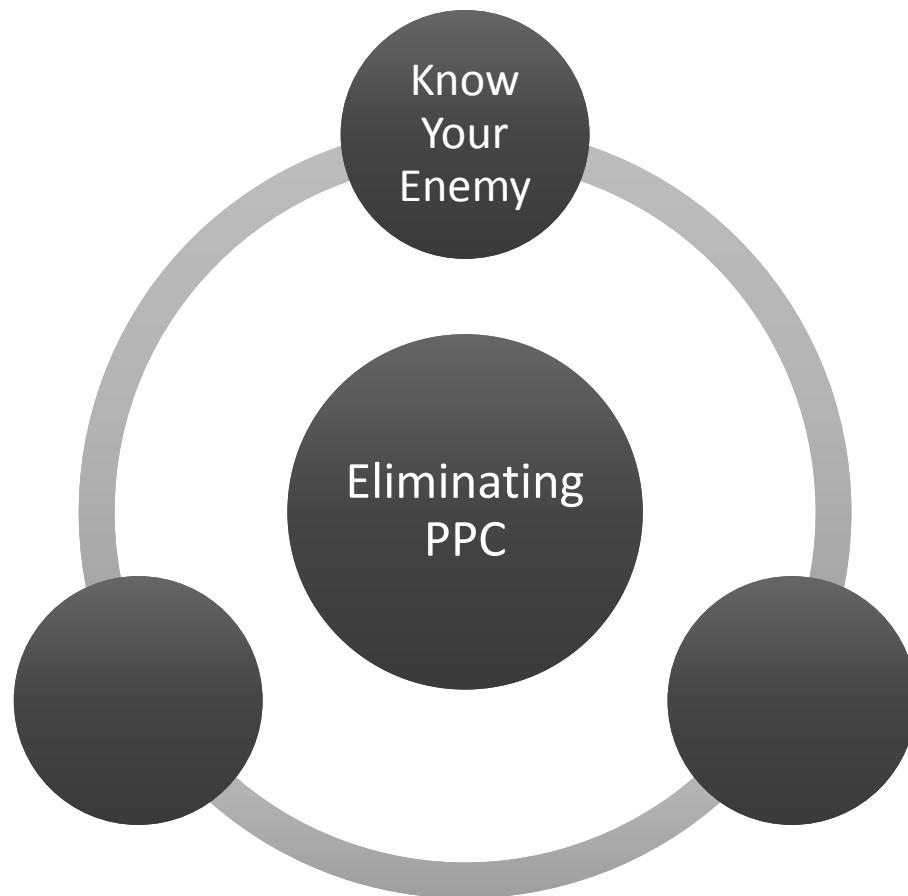
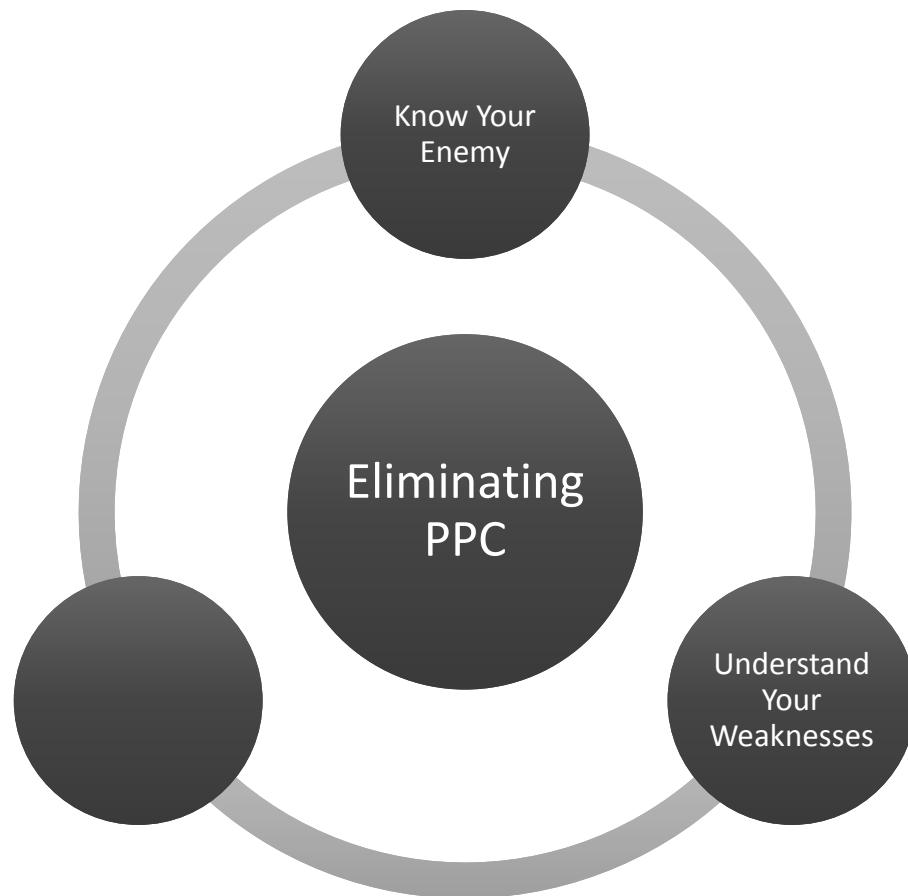


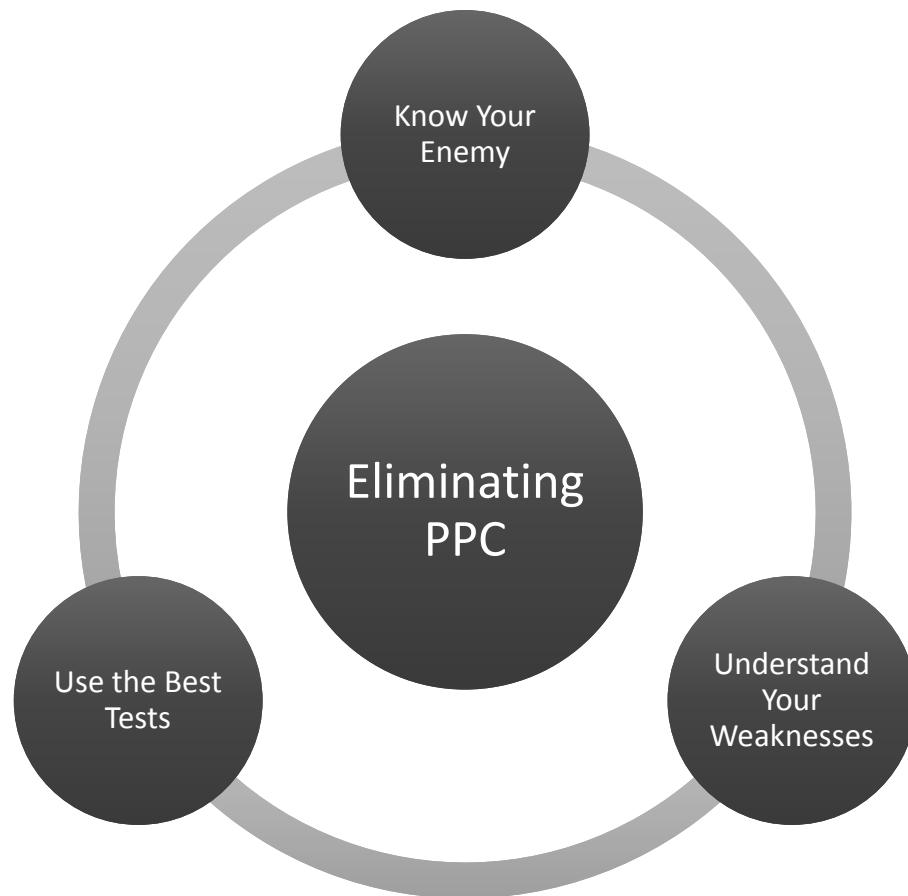
# Detecting and Eliminating Post-Processing Contamination

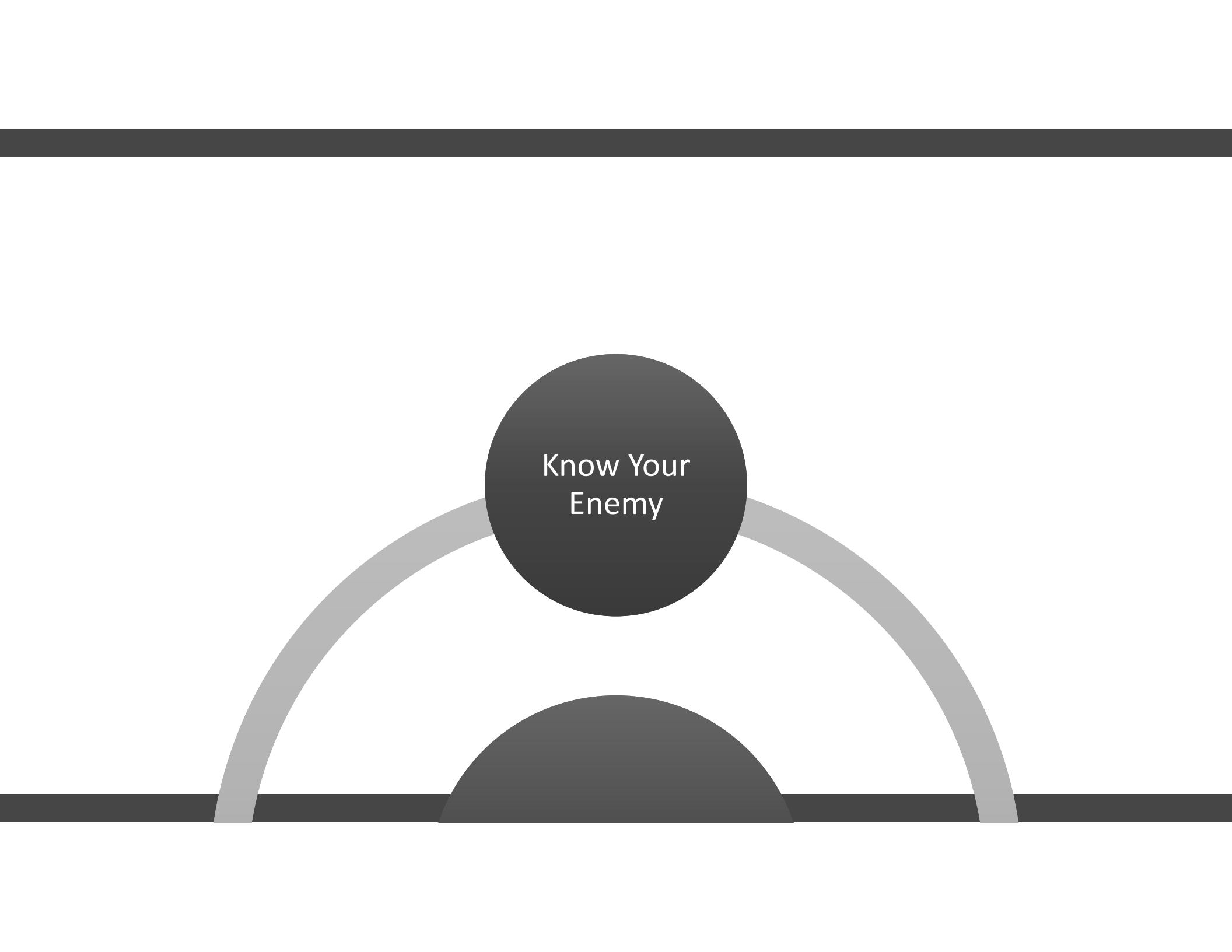
NICOLE MARTIN, MS  
ASSOCIATE DIRECTOR, MILK QUALITY IMPROVEMENT PROGRAM  
DEPARTMENT OF FOOD SCIENCE  
CORNELL UNIVERSITY





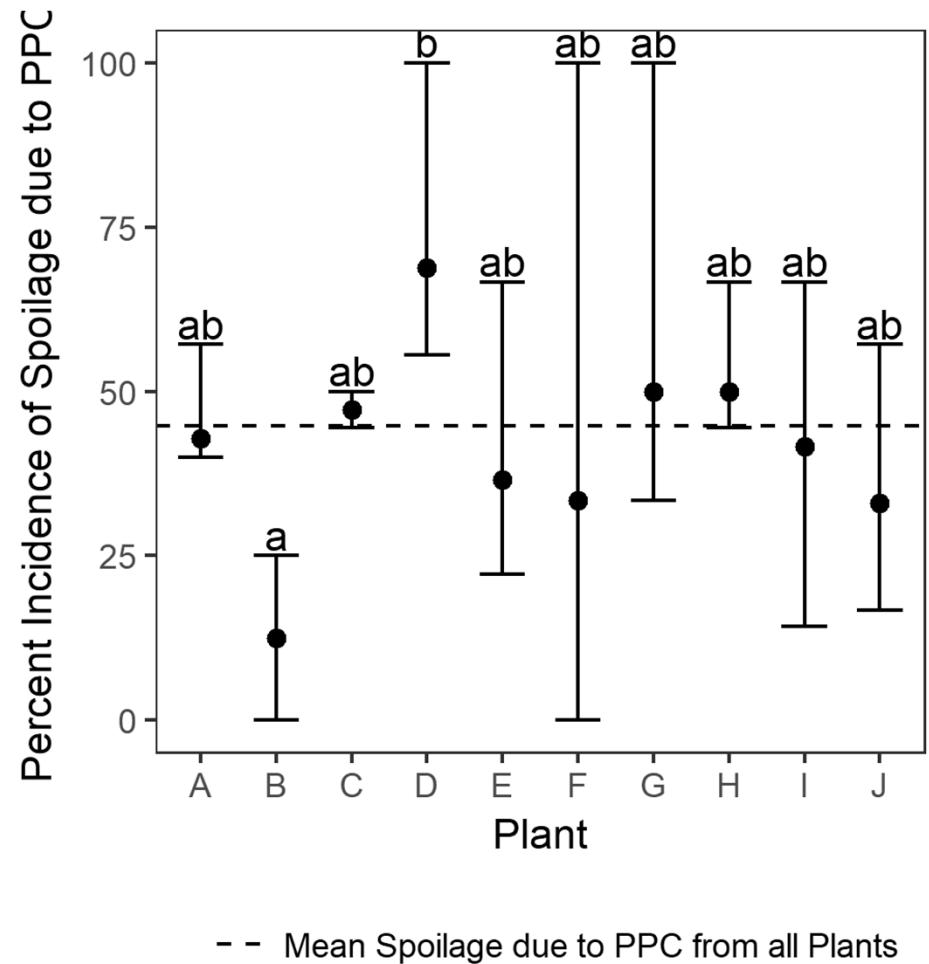






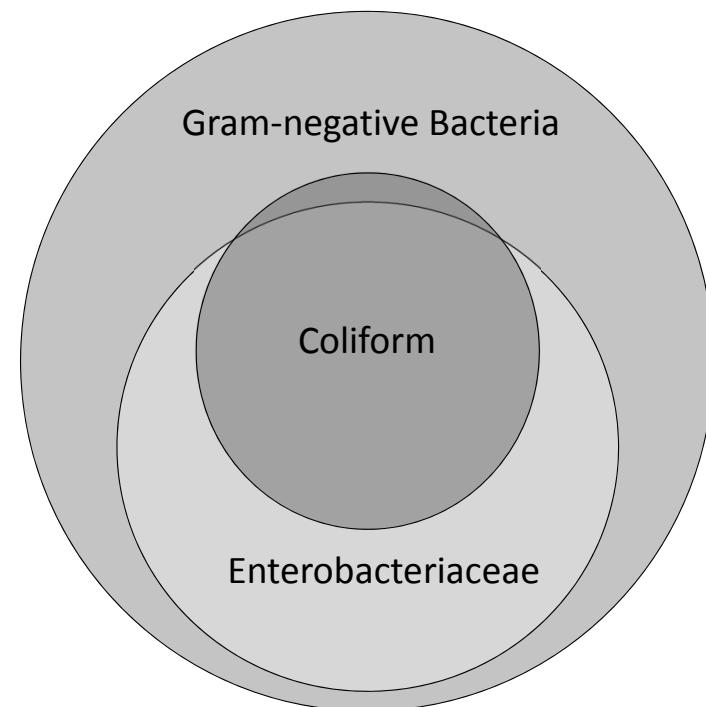
Know Your  
Enemy

Nearly 50% of fluid milk samples show signs of spoilage due to PPC



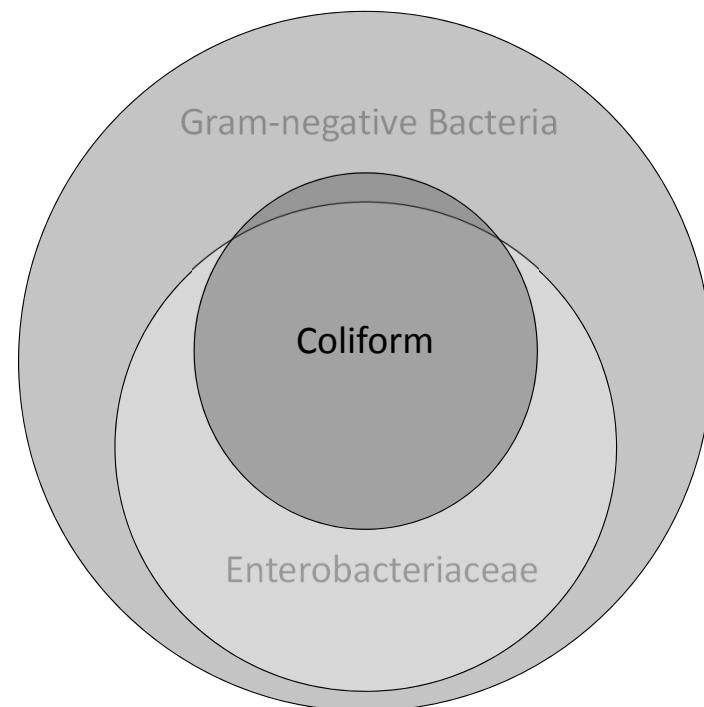
# Post-pasteurization contamination patterns in fluid milk

Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%



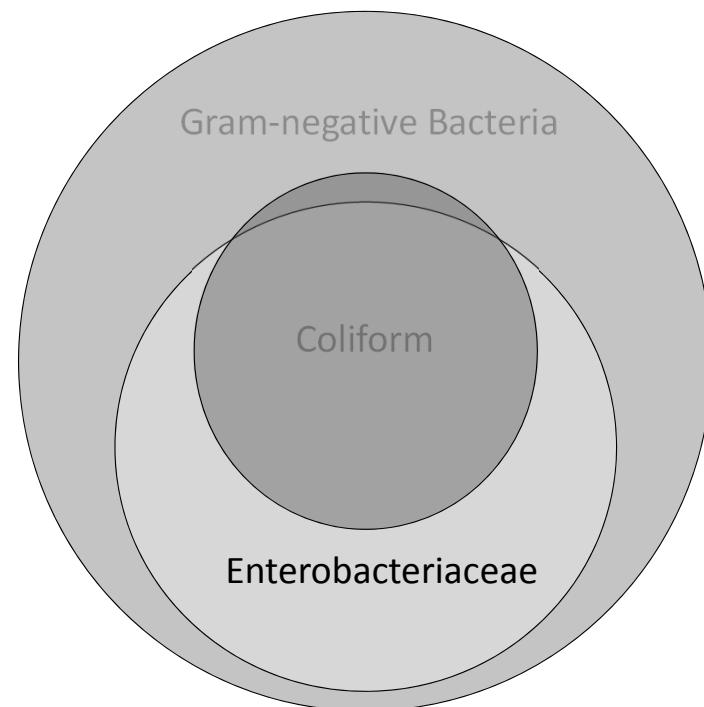
# Post-pasteurization contamination patterns in fluid milk

Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%



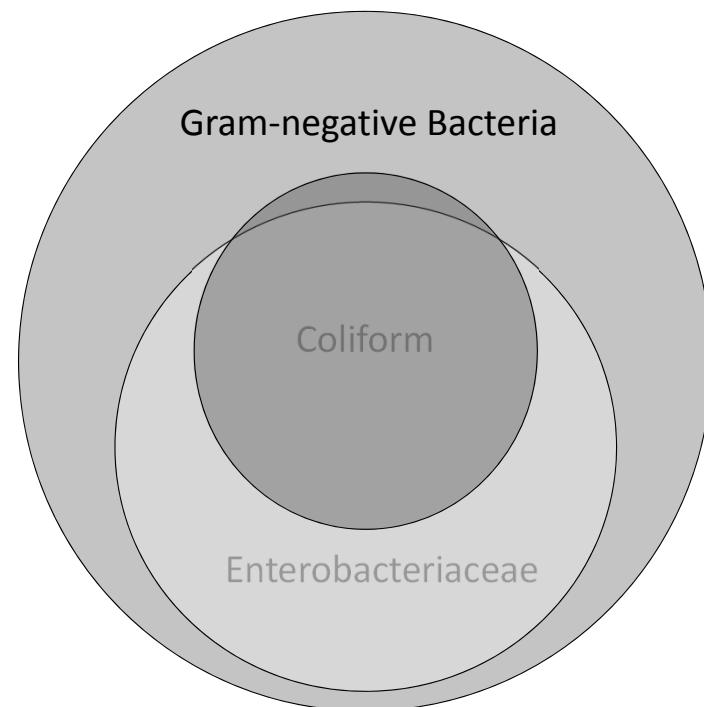
# Post-pasteurization contamination patterns in fluid milk

Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%

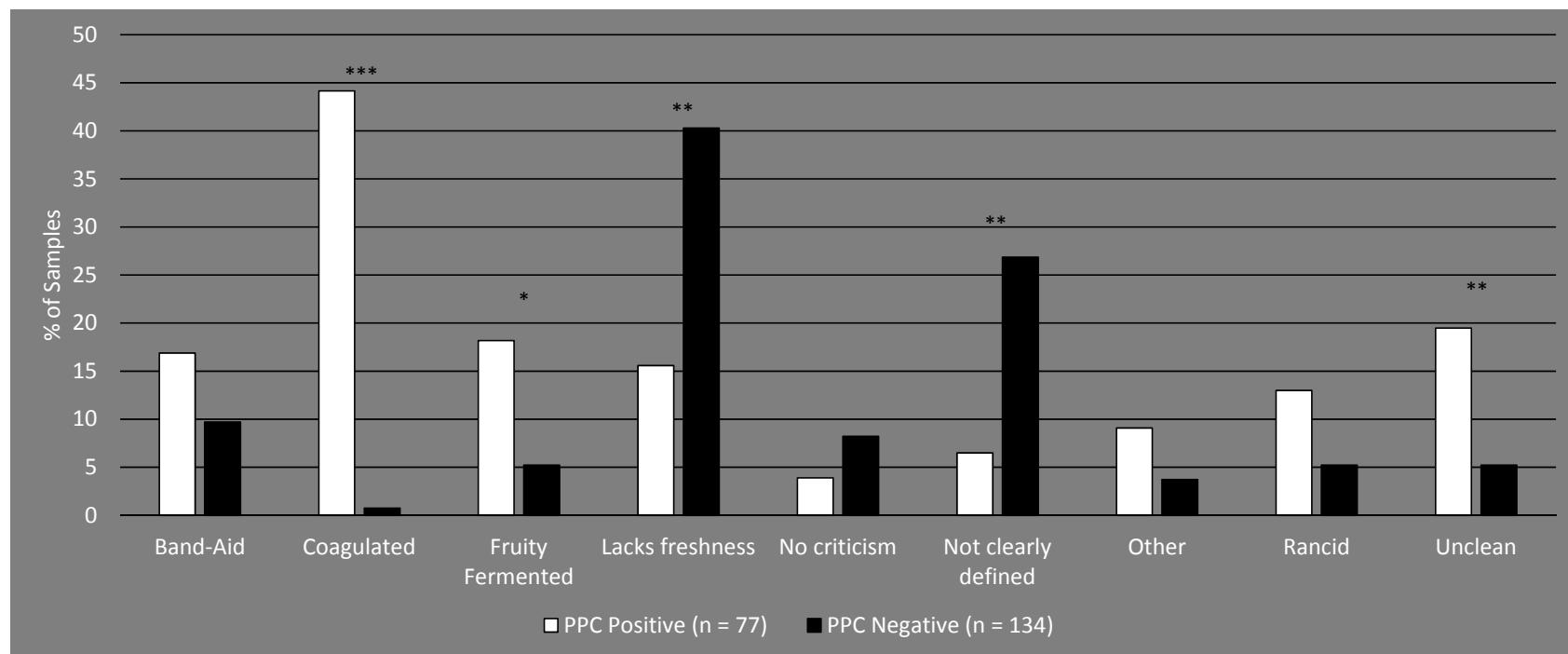


# Post-pasteurization contamination patterns in fluid milk

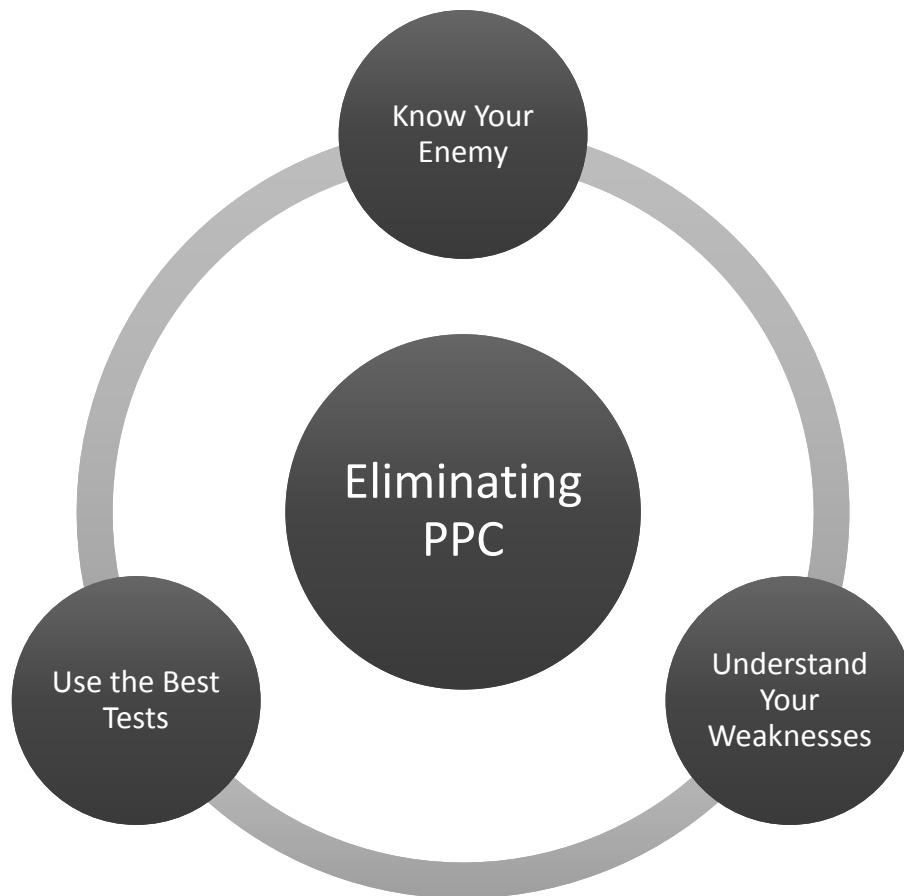
Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%

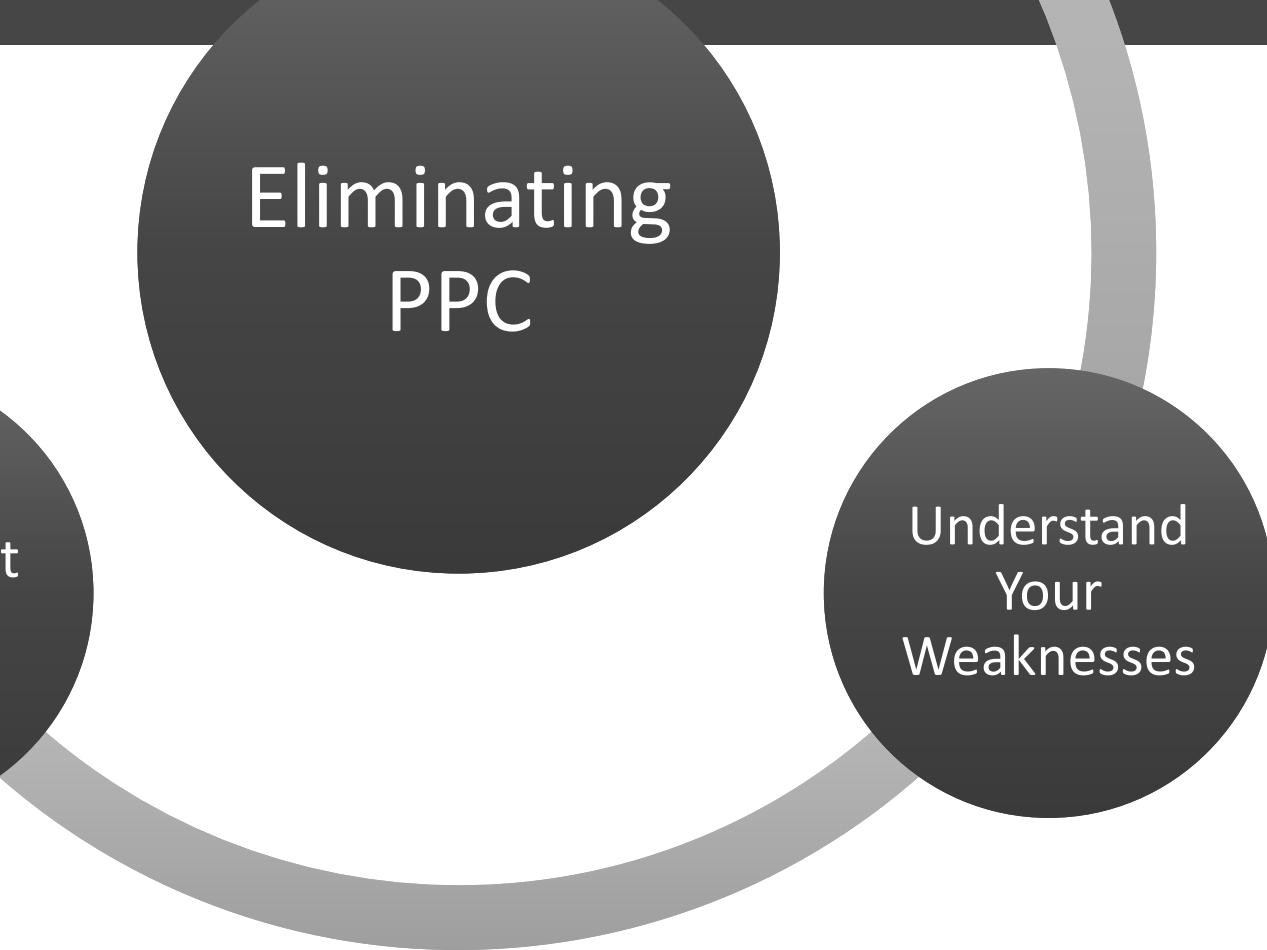


# Post-pasteurization contamination – a barrier to high quality fluid milk



Reichler et al., unpublished

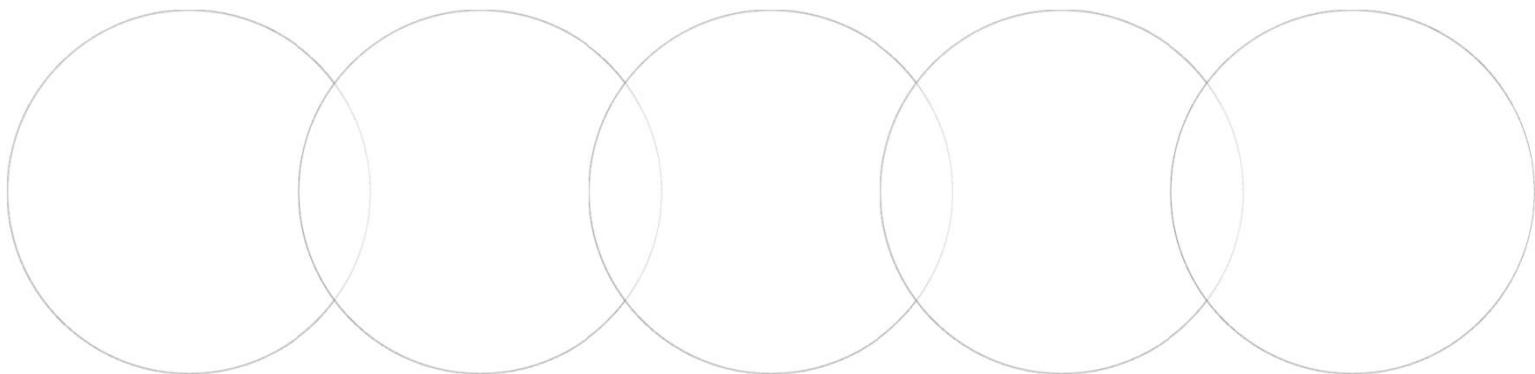




Eliminating  
PPC

Understand  
Your  
Weaknesses

# Understanding Your Weaknesses



# Equipment Design

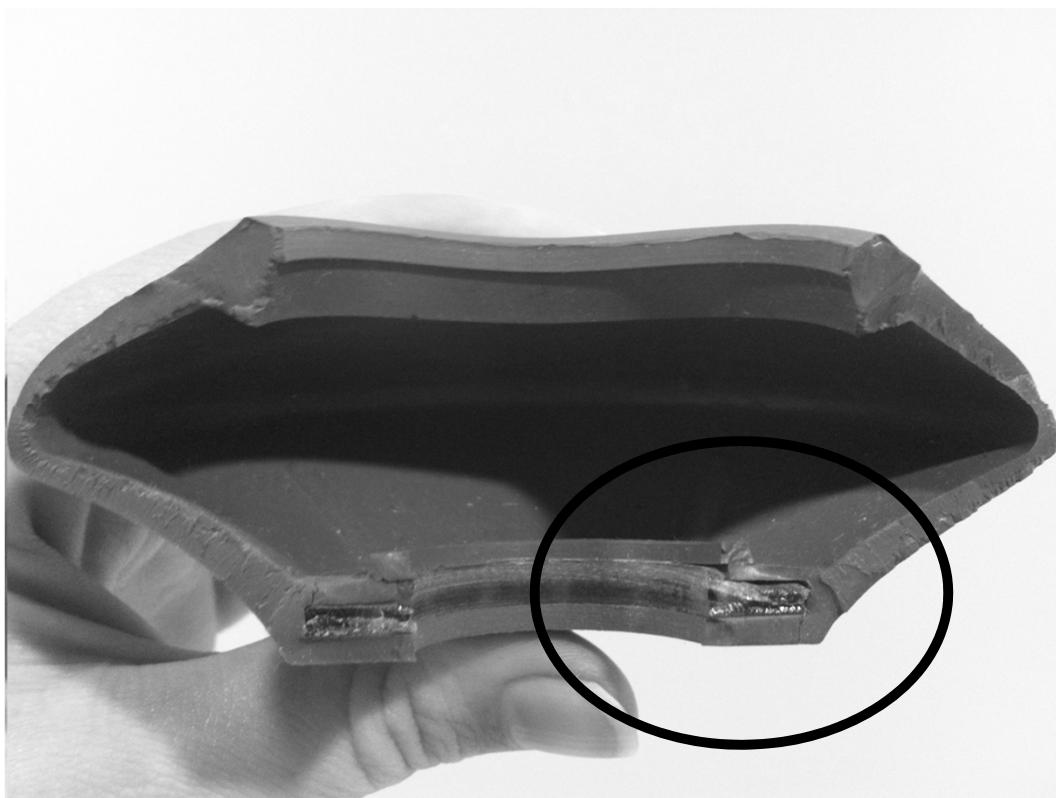
1. Cleanability
2. Construction Materials
3. Accessibility
4. No Liquid Collection
5. Hermetic Sealing
6. No Niches
7. Operational Performance
8. Maintenance Enclosures
9. Hygienic Compatibility
10. Cleaning Validation

## Preventative Maintenance

- Routine schedule and/or as needed
  - Rubber parts
  - Air Valves
  - Spray balls
  - CIP pumps
  - Milk pumps

*\*\*All equipment components should be compatible with cleaning and sanitizing regimes*

# Preventative Maintenance



## Cleaning and Sanitation

- Understand your product (type of soil)
- Water quality matters
- Measure accurately (applies to all methods)
- Verify chemical concentrations using test kits
- Understand chemical interactions
- pH range of efficacy
- Appropriate coverage and contact time
- Temperature
- Cost
- Record Keeping

## Employee Behaviors

- Prevent water and other liquids from becoming stagnant
- Avoid creation of aerosols
  - High pressure water should never be used to clean drains, minimal use in all locations
- Facilitate employee handwashing
- Avoid contact with equipment that has been cleaned and sanitized
  - Re-sanitize if necessary
- Restrict unauthorized personnel from entering processing area

*Understand contamination  
patterns – transient vs.  
persistent contaminants*



	Repeat Isolation	Milk Type	Filter	Container	Size	Date Processed
3		2%	FED-2	Plastic	1/2 Gal	
4		Skim	FED-2	Plastic	1/2 Gal	
5		LF Choc	FED-2	Plastic	1/2 Gal	
6		2%	FOGG-4	Plastic	Gal	
7		LF Choc	FOGG-4	Plastic	Gal	
8		NB-1	Paper	1/2 Pint		2015-08-03
9		LF Choc	NB-2	Paper	1/2 Pint	
10		2%	FED-2	Plastic	1/2 Gal	
11		Skim	FED-2	Plastic	1/2 Gal	
12		LF Choc	FED-2	Plastic	1/2 Gal	
13	X	X				
14						
15	X	X	X			
16						
17						
18	X					
19						
20						
21	X	X	X			
22						
23						
24						
25	X	X	X			
26						
27	X					
28	X					
29	X					
30						
31	X					
32	X					
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Genus

*Limnohabitans*

*Pseudomonas*

*Pseudomonas*

*Pseudomonas*

*Pseudomonas*

*Pseudomonas*

*Pseudomonas*

*Lelliottia*

*Raoultella*

*Pseudomonas*

*Pantoea*

*Pseudomonas*

*Pantoea*

*Kluyvera*

*Raoultella*

*Psychrobacter*

*Acinetobacter*

*Klebsiella*

*Ewingella*

*Stenotrophomonas*

*Pseudomonas*

*Pseudomonas*

*Curvibacter*

*Rahnella*

*Pseudomonas*

*Cerratin*

X

	Repeat Isolation	Milk Type	Filler Container	Size	Date Processed
•	9	X	2%	1	Plastic 1/2 Gal
	22	X	2%	1	Plastic 1/2 Gal
		X	Skim	1	Plastic qt
		X	Skim	2	Plastic 1/2 Pint
		X	LF Choc	2	Plastic 1/2 Pint
		X	LF Choc	2	Plastic 1/2 Pint
					2015-08-11
					2015-10-27
					2016-02-09
					2016-05-24
*	41				
	53				
	71				
	72				
	100				
					Genus
					<i>Pseudomonas</i>
					<i>Pseudomonas</i>
		X			<i>Stenotrophomonas</i>
		X			<i>Enterobacter</i>
					<i>Enterobacter/Kluyvera/Raoultella</i> <sup>1</sup>
			X		<i>Massilia</i>
			X		<i>Pseudomonas</i>

		Repeat Isolation	Milk Type	Filler Container	Size	Date Processed	
•	9	X	2%	1	Plastic	1/2 Gal	
	22	X	2%	1	Plastic	1/2 Gal	
	41		Skim	1	Plastic	qt	
	53	X	Skim	2	Plastic	1/2 Pint	
	71	X	LF Choc	2	Plastic	1/2 Pint	
	72	X	LF Choc	2	Plastic	1/2 Pint	
	100						
2015-08-11							
2015-10-27							
2016-02-09							
2016-05-24							
							Genus
							<i>Pseudomonas</i>
							<i>Pseudomonas</i>
							<i>Stenotrophomonas</i>
							<i>Enterobacter</i>
							<i>Enterobacter/Kluyvera/Raoultella</i> <sup>1</sup>
							<i>Massilia</i>
							<i>Pseudomonas</i>

		Repeat Isolation	Milk Type	Filler	Container	Size	Date Processed
•	1		2%	N8	Paper	1/2 Pint	
•	2		2%	FED-2	Plastic	1/2 Gal	
•	3	X	Skim	FED-2	Plastic	1/2 Gal	
•	4	X	LF Choc	FED-2	Plastic	1/2 Gal	
•	5	X	LF Choc	PET	Plastic	pt	
•	6		2%	Fogg	Plastic	Gal	
•	7		Skim	Fogg	Plastic	Gal	
•	8		LF Choc	Fogg	Plastic	Gal	
•	9			2%	FED-2	Plastic	1/2 Gal
•	10			X	2%	FED-2	Plastic
•	11			X	Skim	FED-2	Plastic
•	12			X	Skim	PET	pt
•	13	X		LF Choc	PET	pt	
•	14	X		LF Choc	PET	pt	
•	15	X		2%	Fogg	Plastic	
•	16	X		Skim	Fogg	Plastic	
•	17	X		LF Choc	Fogg	Plastic	
•	18	X			2%	FED-2	Plastic
•	19	X			X	2%	FED-2
•	20	X			X	Skim	FED-2
•	21	X			X	Skim	PET
•	22	X			X	LF Choc	PET
•	23	X			X	LF Choc	Fogg
•	24	X			X	2%	Fogg
•	25	X			X	Skim	Plastic
•	26	X			X	LF Choc	Gal
•	27	X			X	LF Choc	pt
•	28	X			X	2%	FED-2
•	29	X			X	Skim	Plastic
•	30	X			X	LF Choc	Gal
•	31	X			X	LF Choc	pt
•	32	X			X	2%	FED-2
•	33	X			X	Skim	Plastic
•	34	X			X	LF Choc	Gal
•	35	X			X	LF Choc	pt
•	36	X			X	2%	Fogg
•	37	X			X	Skim	Plastic
•	38	X			X	LF Choc	Gal
•	39	X			X	LF Choc	pt
•	40	X			X	2%	FED-2
•	41	X			X	Skim	Plastic
•	42	X			X	LF Choc	Gal
•	43	X			X	LF Choc	pt
•	44	X			X	2%	Fogg
•	45	X			X	Skim	Plastic
•	46	X			X	LF Choc	Gal
•	47	X			X	LF Choc	pt
•	48	X			X	2%	FED-2
•	49	X			X	Skim	Plastic
•	50	X			X	LF Choc	Gal
•	51	X			X	LF Choc	pt
•	52	X			X	2%	Fogg
•	53	X			X	Skim	Plastic
•	54	X			X	LF Choc	Gal
•	55	X			X	LF Choc	pt
•	56	X			X	2%	FED-2
•	57	X			X	Skim	Plastic
•	58	X			X	LF Choc	Gal
•	59	X			X	LF Choc	pt
•	60	X			X	2%	Fogg
•	61	X			X	Skim	Plastic
•	62	X			X	LF Choc	Gal
•	63	X			X	LF Choc	pt
•	64	X			X	2%	FED-2
•	65	X			X	Skim	Plastic
•	66	X			X	LF Choc	Gal
•	67	X			X	LF Choc	pt
•	68	X			X	2%	Fogg
•	69	X			X	Skim	Plastic
•	70	X			X	LF Choc	Gal
•	71	X			X	LF Choc	pt
•	72	X			X	2%	FED-2
•	73	X			X	Skim	Plastic
•	74	X			X	LF Choc	Gal
•	75	X			X	LF Choc	pt
•	76	X			X	2%	Fogg
•	77	X			X	Skim	Plastic
•	78	X			X	LF Choc	Gal
•	79	X			X	LF Choc	pt
•	80	X			X	2%	FED-2
•	81	X			X	Skim	Plastic
•	82	X			X	LF Choc	Gal
•	83	X			X	LF Choc	pt
•	84	X			X	2%	Fogg
•	85	X			X	Skim	Plastic
•	86	X			X	LF Choc	Gal
•	87	X			X	LF Choc	pt
•	88	X			X	2%	FED-2
•	89	X			X	Skim	Plastic
•	90	X			X	LF Choc	Gal
•	91	X			X	LF Choc	pt
•	92	X			X	2%	Fogg
•	93	X			X	Skim	Plastic
•	94	X			X	LF Choc	Gal
•	95	X			X	LF Choc	pt
•	96	X			X	2%	FED-2
•	97	X			X	Skim	Plastic
•	98	X			X	LF Choc	Gal
•	99	X			X	LF Choc	pt
•	100	X			X	2%	Fogg
•	101	X			X	Skim	Plastic
•	102	X			X	LF Choc	Gal
•	103	X			X	LF Choc	pt
•	104	X			X	2%	FED-2
•	105	X			X	Skim	Plastic
•	106	X			X	LF Choc	Gal
•	107	X			X	LF Choc	pt
•	108	X			X	2%	Fogg
•	109	X			X	Skim	Plastic
•	110	X			X	LF Choc	Gal
•	111	X			X	LF Choc	pt
•	112	X			X	2%	FED-2
•	113	X			X	Skim	Plastic

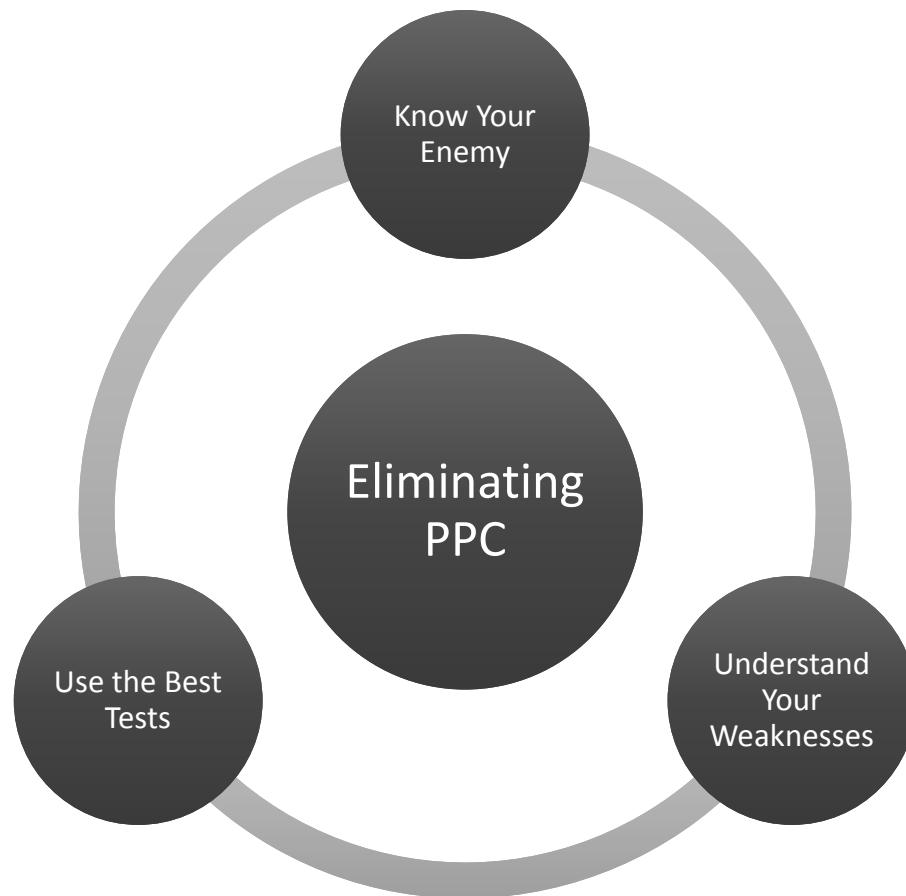
## Genus

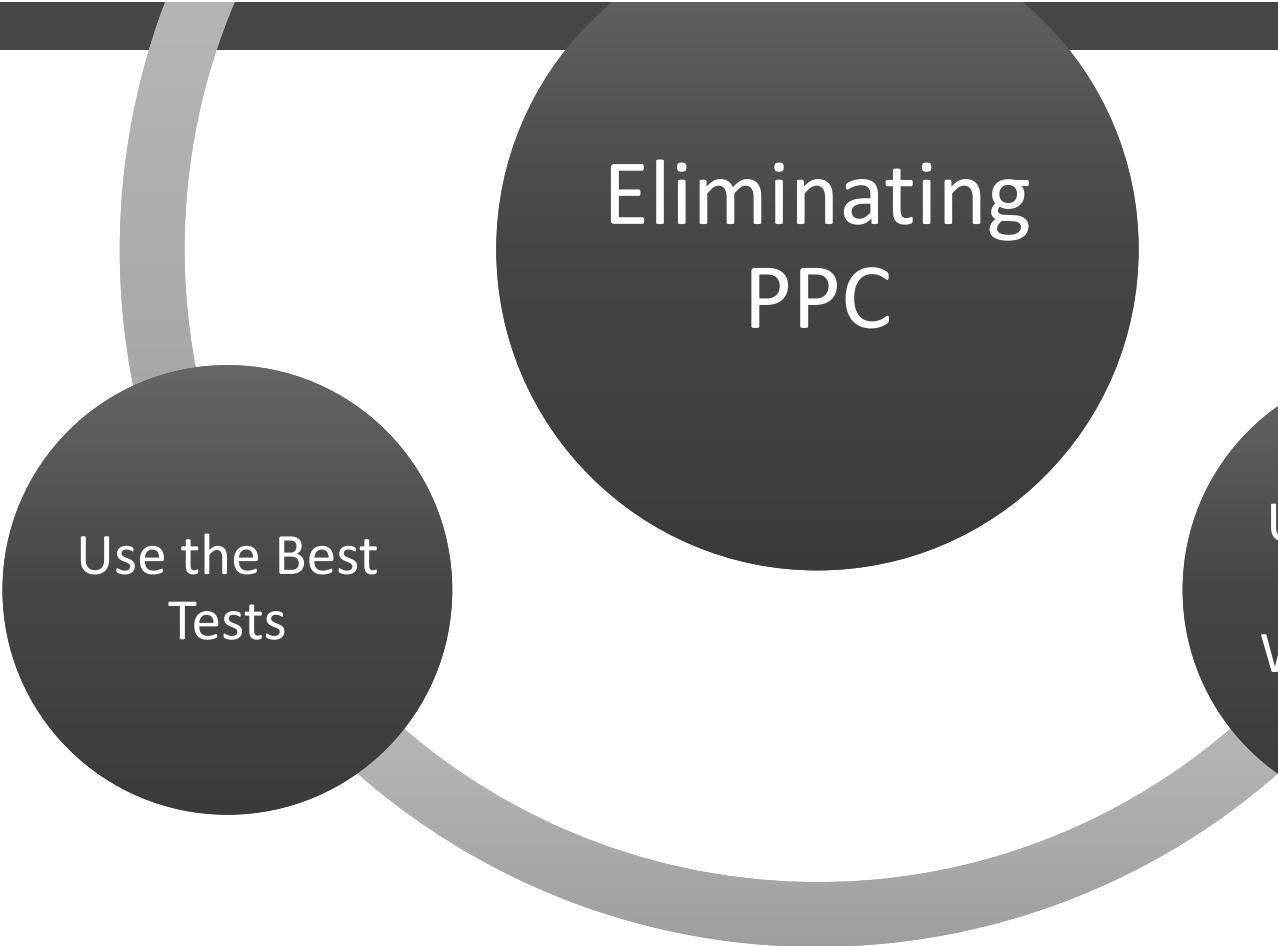
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*Acinetobacter*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Stenotrophomonas*  
*Pseudomonas*  
*Obesumbacterium/Ewingella*  
*Pseudomonas*  
*Klebsiella*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Pseudomonas*  
*Citrobacter*

	Repeat Isolation	Milk Type	Filler	Container	Size	Date Processed	
*	1	2%	N8	Paper	1/2 Pint	2015-08-03	
*	2	2%	FED-2	Plastic	1/2 Gal		
*	3	Skim	FED-2	Plastic	1/2 Gal		
*	4	lF Choc	FED-2	Plastic	1/2 Gal		
*	5	lF Choc	PET	Plastic	pt		
*	6	2%	Fogg	Plastic	Gal		
*	7	Skim	Fogg	Plastic	Gal		
*	8	lF Choc	Fogg	Plastic	Gal		
*	9	2%	FED-2	Plastic	1/2 Gal	2015-10-05	
*	10	2%	FED-2	Plastic	qt		
*	11	Skim	FED-2	Plastic	qt		
*	12	Skim	PET	Plastic	pt		
*	13	lF Choc	PET	Plastic	pt		
*	14	lF Choc	PET	Plastic	pt		
*	15	2%	Fogg	Plastic	Gal		
*	16	Skim	Fogg	Plastic	Gal		
*	17	lF Choc	Fogg	Plastic	Gal		
*	18	2%	FED-2	Plastic	qt	2016-02-08	
*	19	Skim	FED-2	Plastic	qt		
*	20	2%	FED-2	Plastic	1/2 Gal		
*	21	Skim	PET	Plastic	pt		
*	22	lF Choc	PET	Plastic	pt		
*	23	2%	Fogg	Plastic	Gal		
*	24	Skim	Fogg	Plastic	Gal		
*	25	lF Choc	Fogg	Plastic	Gal		
*	26	2%	FED-2	Plastic	qt	2016-04-18	
*	27	Skim	FED-2	Plastic	1/2 Gal		
*	28	Skim	PET	Plastic	pt		
*	29	lF Choc	PET	Plastic	pt		
*	30	Choc	PET	Plastic	pt		
*	31	2%	Fogg	Plastic	Gal		
*	32	Skim	Fogg	Plastic	Gal		
*	33	lF Choc	Fogg	Plastic	Gal		
*	34	X	X	X	X		
*	35	X	X	X	X		
*	36	X	X	X	X		
*	37	X	X	X	X		
*	38	X	X	X	X		
*	39	X	X	X	X		
*	40	X	X	X	X		
*	41	X	X	X	X		
*	42	X	X	X	X		
*	43	X	X	X	X		
*	44	X	X	X	X		
*	45	X	X	X	X		
*	46	X	X	X	X		
*	47	X	X	X	X		
*	48	X	X	X	X		
*	49	X	X	X	X		
*	50	X	X	X	X		
*	51	X	X	X	X		
*	52	X	X	X	X		
*	53	X	X	X	X		
*	54	X	X	X	X		
*	55	X	X	X	X		
*	56	X	X	X	X		
*	57	X	X	X	X		
*	58	X	X	X	X		
*	59	X	X	X	X		
*	60	X	X	X	X		
*	61	X	X	X	X		
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*	63	X	X	X	X		
*	64	X	X	X	X		
*	65	X	X	X	X		
*	66	X	X	X	X		
*	67	X	X	X	X		
*	68	X	X	X	X		
*	69	X	X	X	X		
*	70	X	X	X	X		
*	71	X	X	X	X		
*	72	X	X	X	X		
*	73	X	X	X	X		
*	74	X	X	X	X		
*	75	X	X	X	X		
*	76	X	X	X	X		
*	77	X	X	X	X		
*	78	X	X	X	X		
*	79	X	X	X	X		
*	80	X	X	X	X		
*	81	X	X	X	X		
*	82	X	X	X	X		
*	83	X	X	X	X		
*	84	X	X	X	X		
*	85	X	X	X	X		
*	86	X	X	X	X		
*	87	X	X	X	X		
*	88	X	X	X	X		
*	89	X	X	X	X		
*	90	X	X	X	X		
*	91	X	X	X	X		
*	92	X	X	X	X		
*	93	X	X	X	X		
*	94	X	X	X	X		
*	95	X	X	X	X		
*	96	X	X	X	X		
*	97	X	X	X	X		
*	98	X	X	X	X		
*	99	X	X	X	X		
*	100	X	X	X	X		
*	101	X	X	X	X		
*	102	X	X	X	X		
*	103	X	X	X	X		
*	104	X	X	X	X		
*	105	X	X	X	X		
*	106	X	X	X	X		
*	107	X	X	X	X		
*	108	X	X	X	X		
*	109	X	X	X	X		
*	110	X	X	X	X		
*	111	X	X	X	X		
*	112	X	X	X	X		
*	113	X	X	X	X		

## Genus

*Acinetobacter**Acinetobacter**Pseudomonas**Pseudomonas**Pseudomonas**Pseudomonas**Pseudomonas**Pseudomonas**Stenotrophomonas**Pseudomonas**Obesumbacterium/Ewingella**Pseudomonas**Klebsiella**Pseudomonas**Pseudomonas**Pseudomonas**Pseudomonas**Citrobacter*





Eliminating  
PPC

Use the Best  
Tests

## Use the Best Tests

- May require pre-enrichment for low level contamination
- Gram-negative PPC
  - Total Gram-negative testing (Crystal Violet Tetrazolium Agar)
  - EB
  - Coliform
- Fungi
  - Broad range of media (DRBC, PDA, YM, etc)
    - Antibiotic vs. acidified
  - Enrichment can be challenging

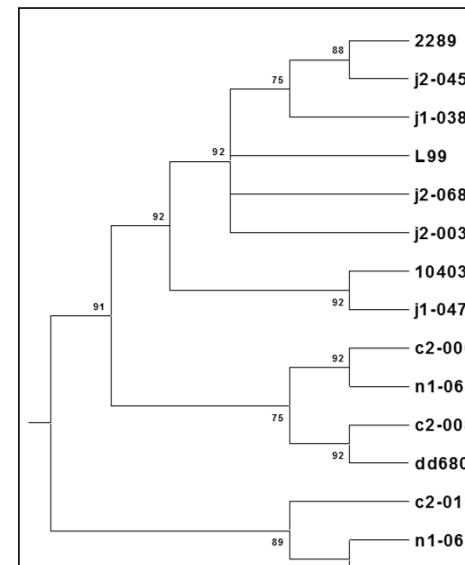
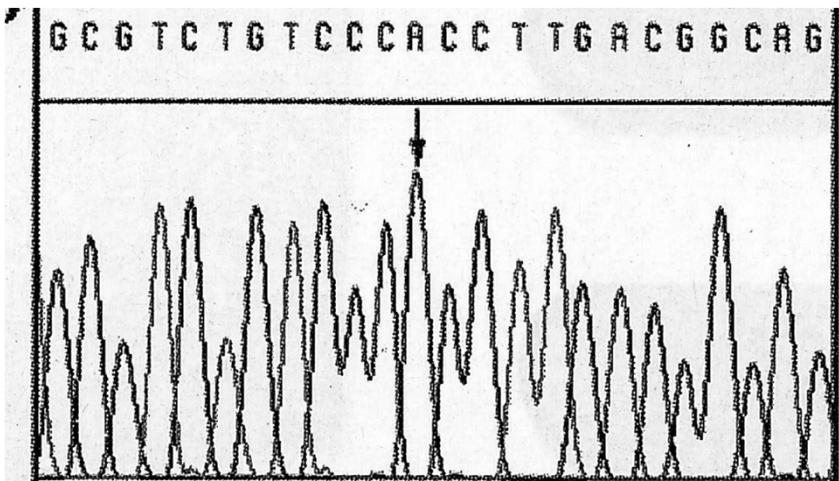
# Rapid detection of post-pasteurization contamination

Media	Pre-Incubation Temperature (°C)	Sensitivity (%)	Specificity (%)
Crystal Violet Tetrazolium (CVTA) Agar	21	70	93
Enterobacteriaceae (EB) Petrifilm	21	23	100
Coliform Petrifilm	21	15	100
Crystal Violet Tetrazolium (CVTA) Agar	13	33	98
Enterobacteriaceae (EB) Petrifilm	13	7	100
Coliform Petrifilm	13	3	100

## Molecular subtyping

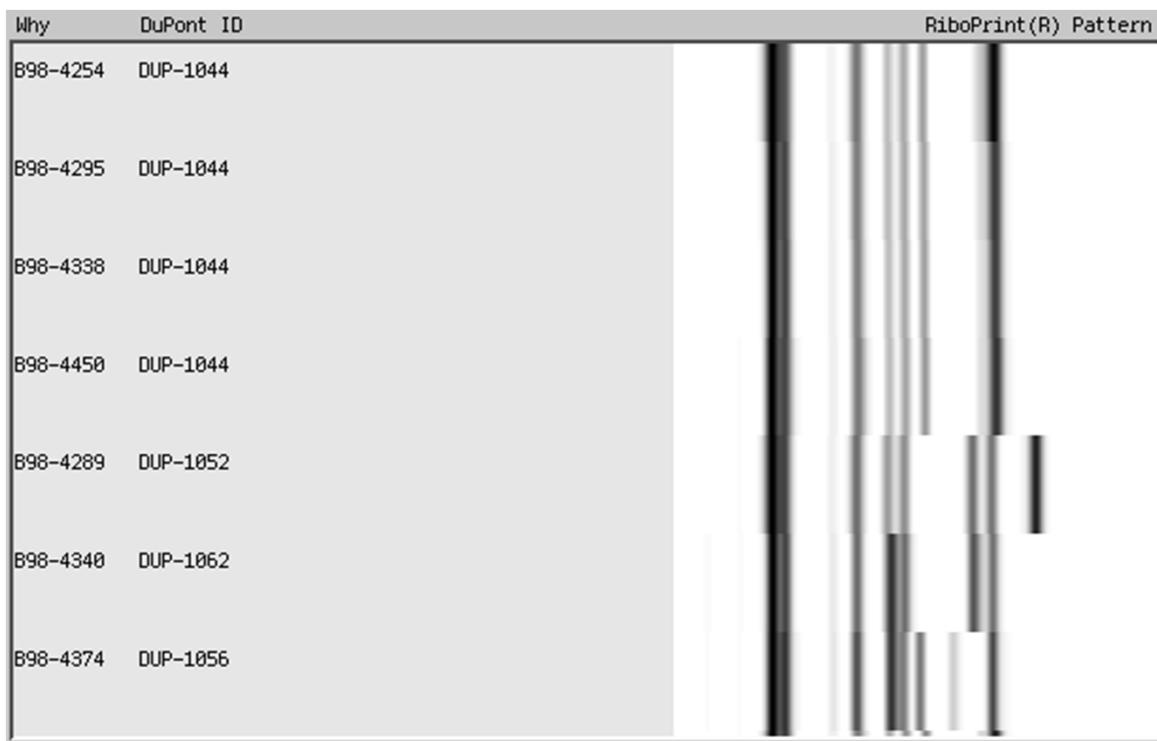
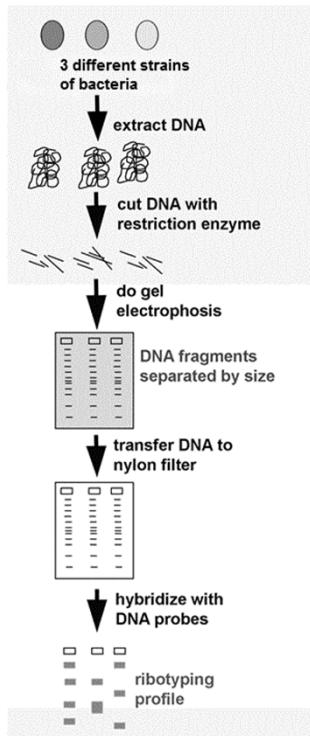
- Essential to understanding source tracking and contamination patterns in food processing facilities
  - DNA sequence-based subtyping
  - Ribotyping
  - Pulsed Field Gel Electrophoresis (PFGE)
  - Whole Genome Sequencing (WGS)

## DNA sequencing-based subtyping

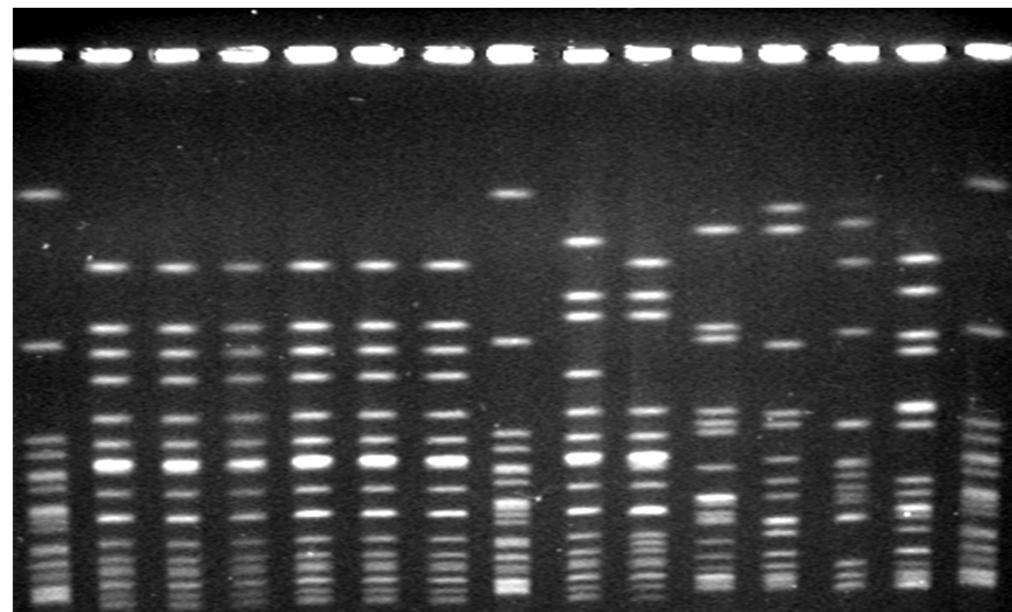
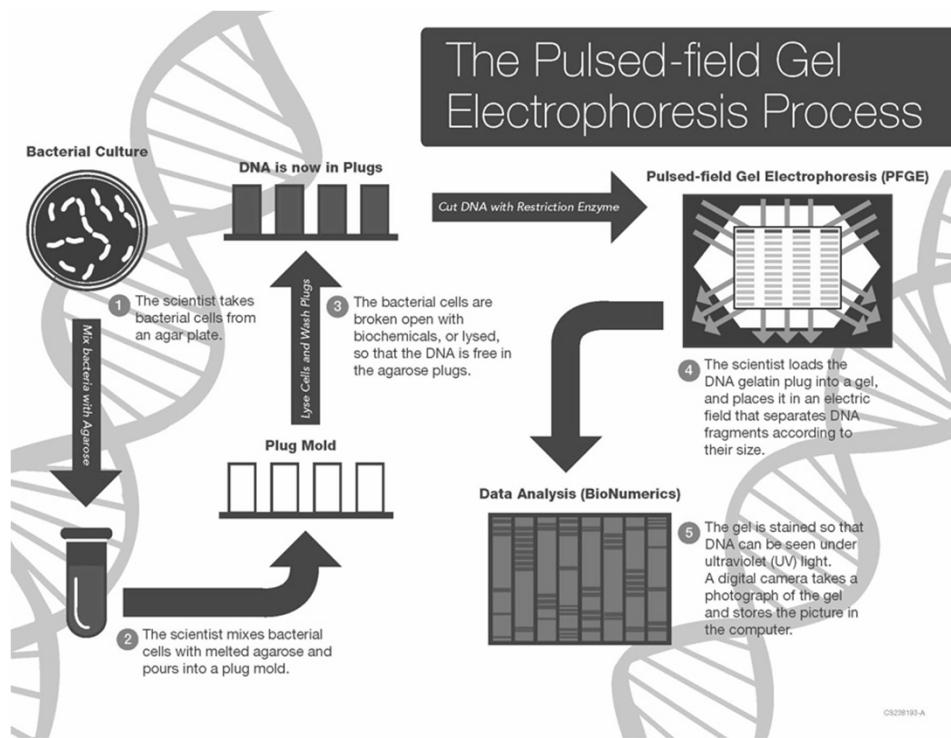


Isolate 1 AACATGCAGACTGACGATTGACGTAGGGCTAGACGTTGACTG  
Isolate 2 AACATGCAGACTGACGATTGCGTAGGGCTAGACGTTGACTG  
Isolate 3 AACATGCAGACTGACGATTGACGTAGGGCTAGACGTTGACTG  
Isolate 4 AACATGCATACTGACGATTGACGAAGGGCTAGACGTTGACTG

# Ribotyping



# Pulsed Field Gel Electrophoresis (PFGE)



<http://www.cdc.gov/pulsenet/pathogens/protocol-images.html#pfge>

# Whole Genome Sequencing (WGS) for improved subtyping



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RESEARCH

## Rapid Whole-Genome Sequencing for Surveillance of *Salmonella enterica* Serovar Enteritidis

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## A Case Study: When Cheese Gets the Blues

- Low salt, low acid cheese ( $\text{pH} \sim 5.9\text{-}6.0$ ) produced at Company X
- Customer complaints trigger source tracking investigation
- Swabs collected from equipment and processing environment
  - *Pseudomonas* found ubiquitously



**Table 3.** Characteristics of selected isolates from Queso Fresco and environmental swabs and comparison isolates from an unrelated study

FSL no. <sup>1</sup>	Isolate ID <sup>2</sup>	Isolate origin (sampling) <sup>3</sup>	Color development on PDA <sup>4</sup>	Fluorescence under UV <sup>5</sup>	Cheese inoculation <sup>6</sup>	Isolate similarity for selected tests <sup>7</sup>	
						PFGE	Ribotype
W5-203	<i>Pseudomonas fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-206	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-207	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-208	<i>P. fluorescens</i>	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5-287	<i>P. fluorescens</i>	PT-2 cheese curd (1)	Blue	Yes	Blue	1	1
W5-296	<i>P. fluorescens</i>	PT-2 cheese curd (1)	Blue	Yes	Blue	2	1
W5-319	<i>P. fluorescens</i>	CV-2 agitator drain tube swab (3)	No color	No	No color	3	1
W5-325	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5-326	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5-327	<i>P. fluorescens</i>	CV-1 agitator track swab (3)	No color	No	No color	3	1
R5-199*	<i>P. fluorescens</i>	Dairy plant isolate	No color	No	No color	NA	1
R5-202*	<i>P. fluorescens</i>	Dairy plant isolate	No color	No	No color	NA	1

## When Cheese Gets the Blues – Lessons Learned

- Molecular subtyping tools allowed for sensitive discrimination between closely related *Pseudomonas* strains
- Implementation of cleaning and sanitation changes and improved equipment design contributed to the elimination of persistent contamination

# Summary

- Know your enemy
  - *Pseudomonas* is one of the most common bacterial agents causing PPC in dairy products
- Understand your weaknesses
  - Preventing PPC requires sanitary equipment design, stringent preventative maintenance, comprehensive cleaning and sanitation programs, improving employee behaviors and understanding contamination patterns
- Use the best tests
  - The best indicator test should be comprehensive and take into consideration product characteristics
  - Subtyping tools are sensitive and powerful in performing root cause analysis for PPC