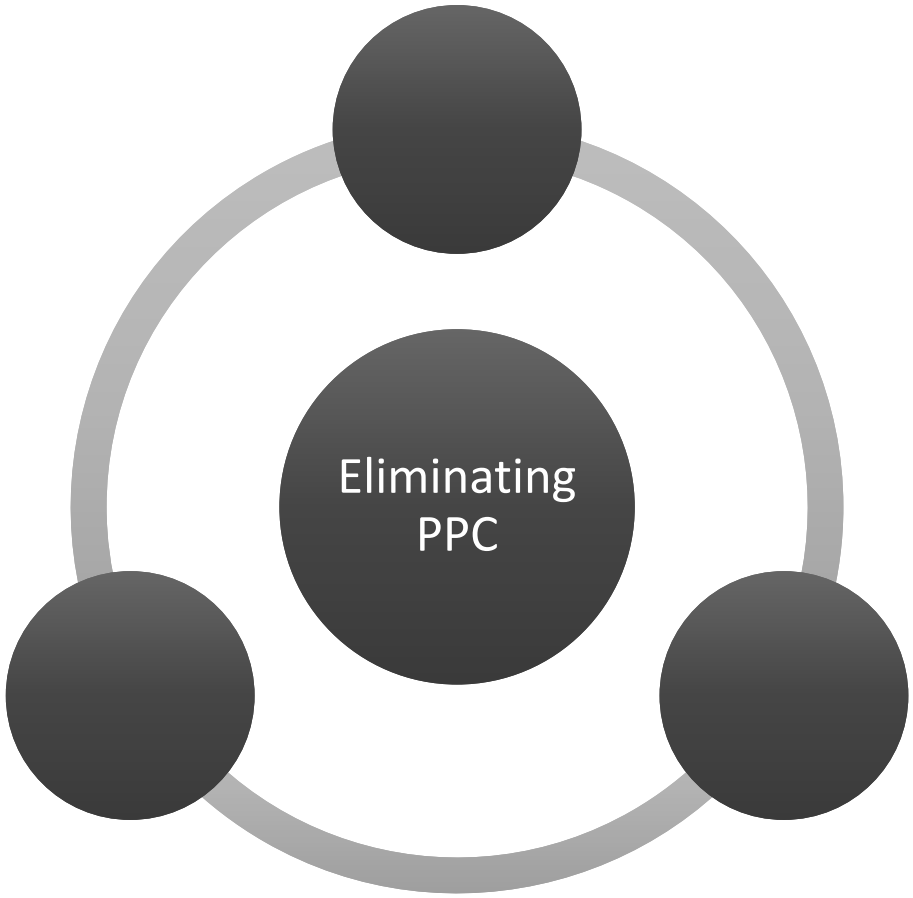
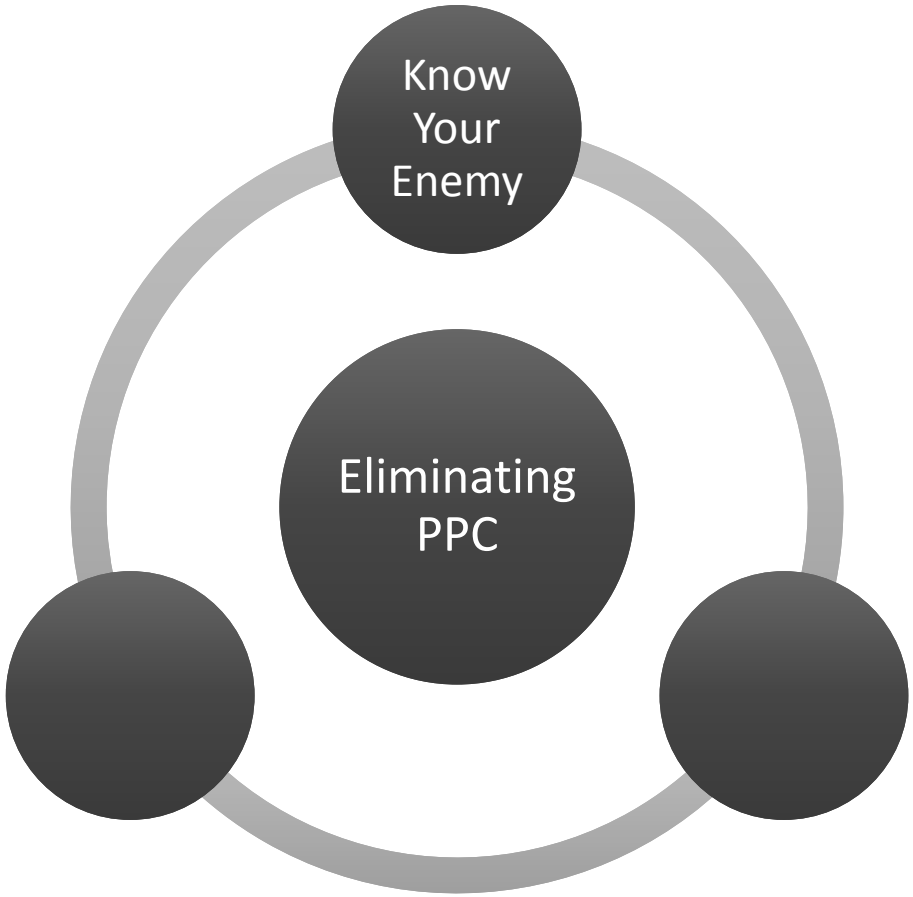
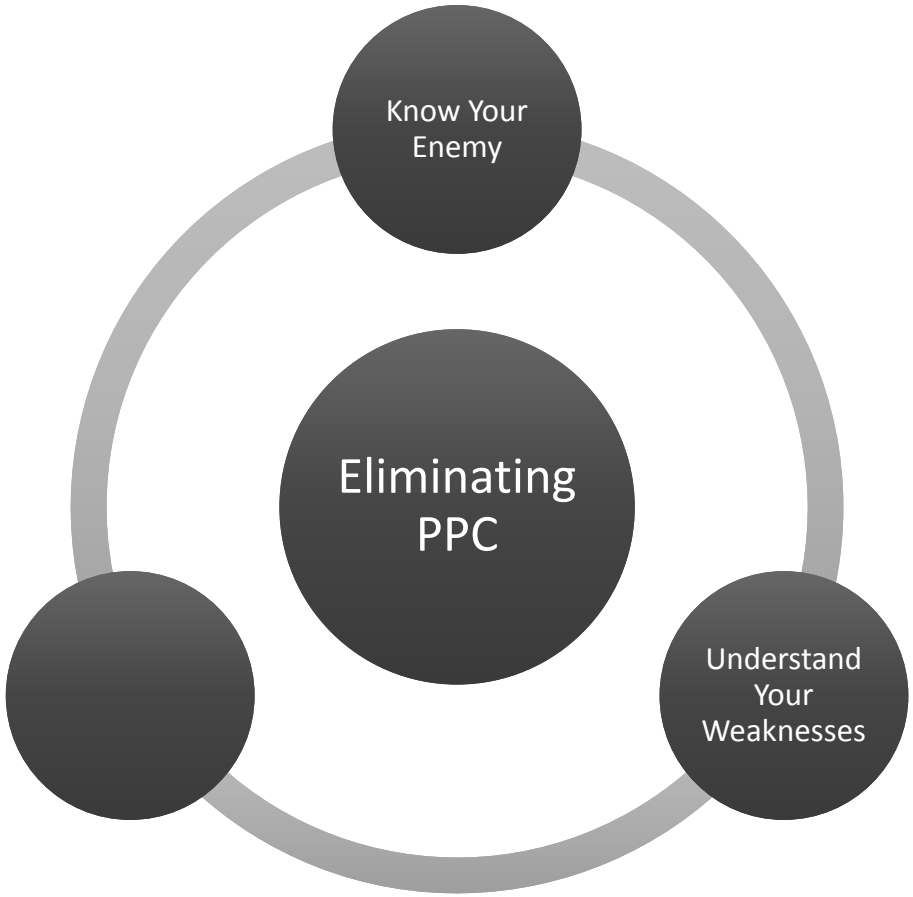
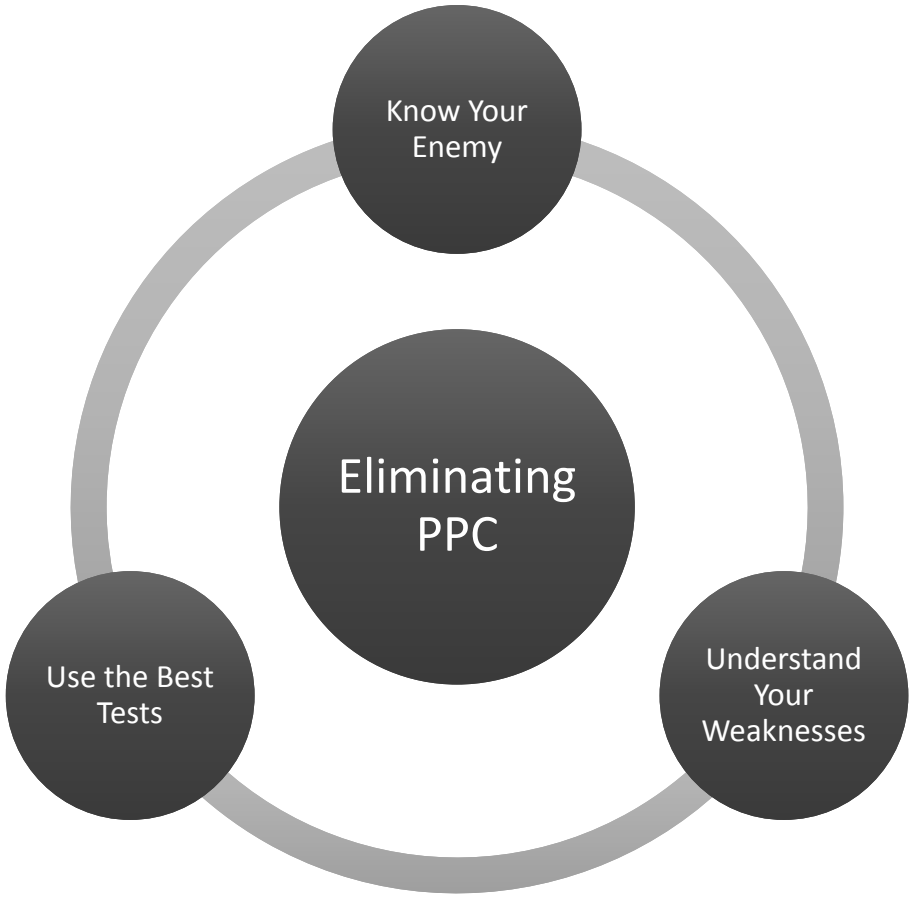
Detecting and Eliminating Post-Processing Contamination

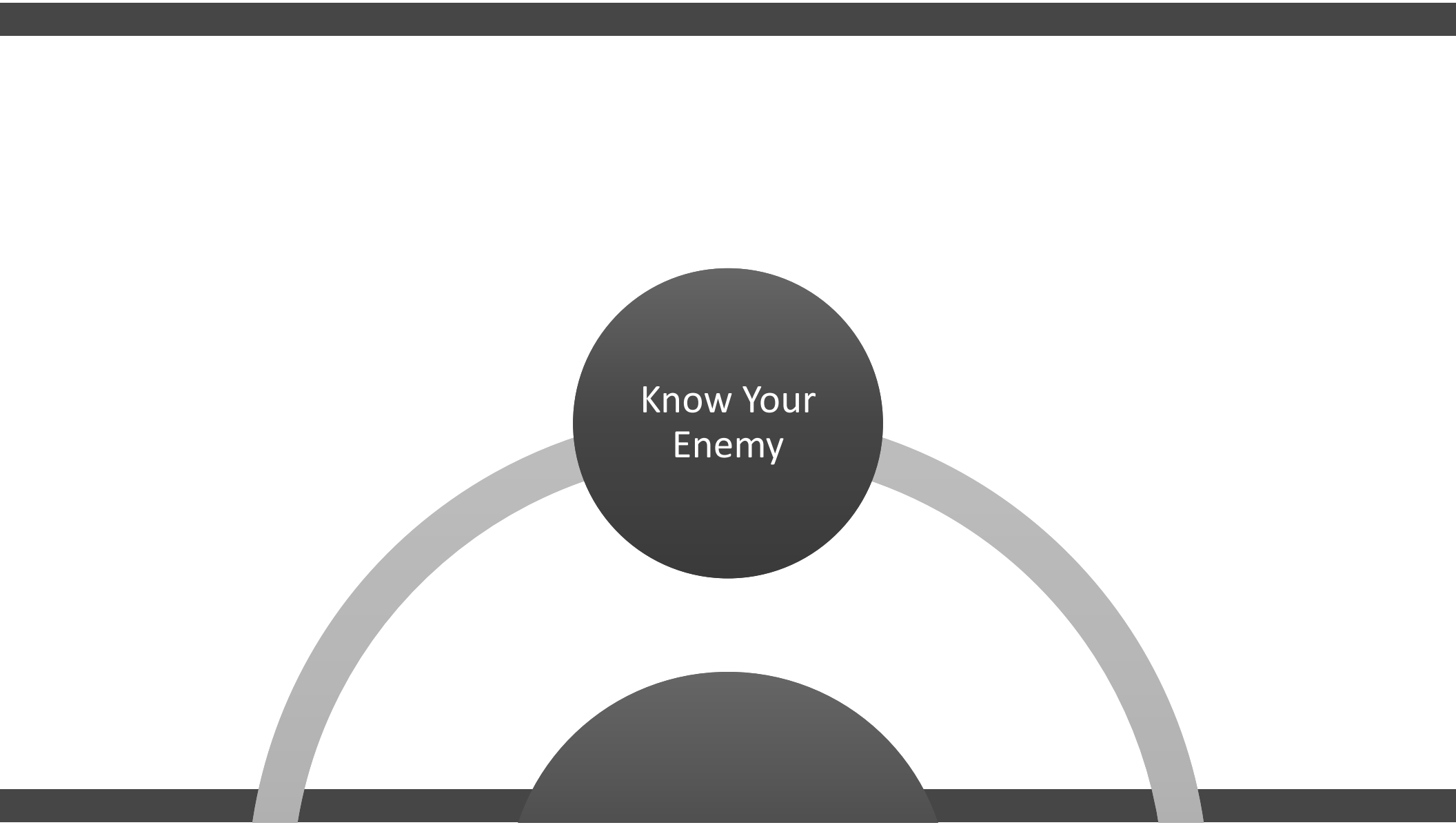
NICOLE MARTIN, MS
ASSOCIATE DIRECTOR, MILK QUALITY IMPROVEMENT PROGRAM
DEPARTMENT OF FOOD SCIENCE
CORNELL UNIVERSITY


Eliminating
PPC

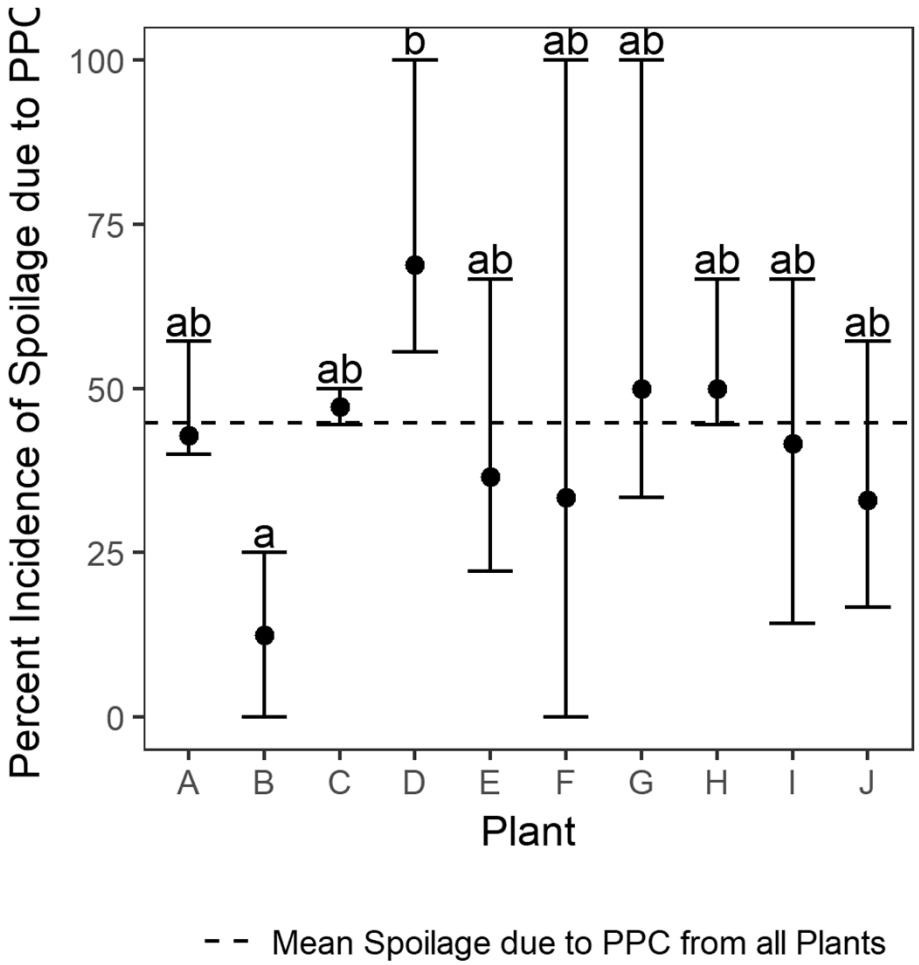

Know
Your
Enemy
Eliminating
PPC


Know Your Enemy
Eliminating PPC
Understand Your Weaknesses


Know Your Enemy
Eliminating PPC
Use the Best Tests
Understand Your Weaknesses


Know Your
Enemy

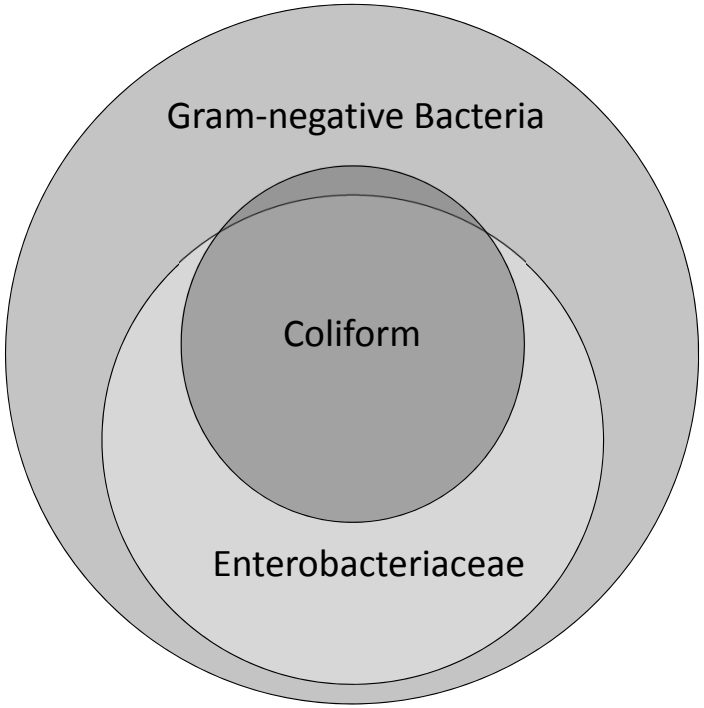
Nearly 50% of fluid milk samples show signs of spoilage due to PPC



Reichler et al., unpublished

Post-pasteurization contamination patterns in fluid milk

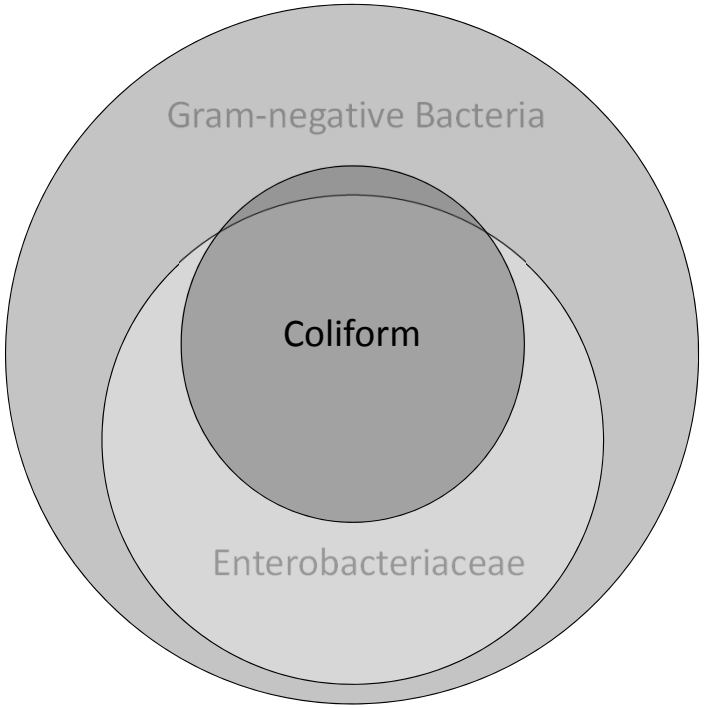
Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%



Alles et al., submitted

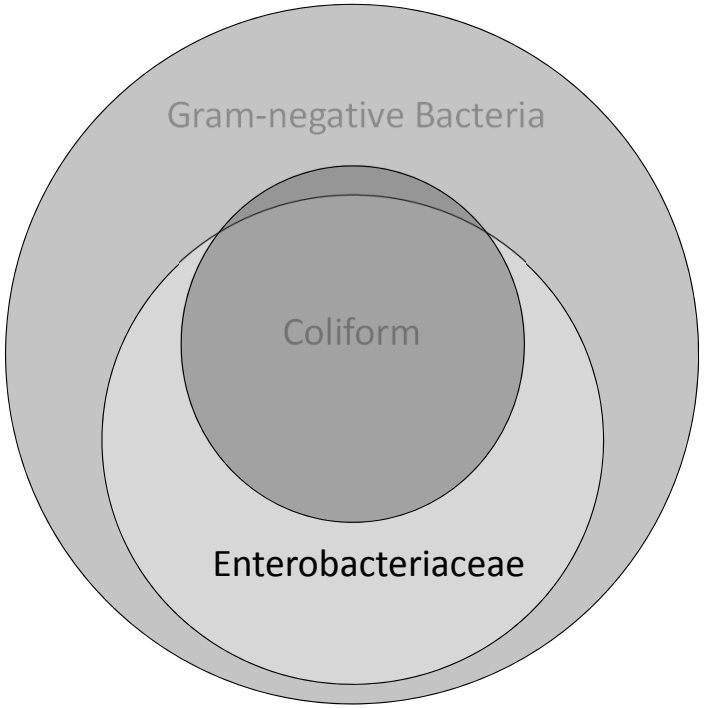
Post-pasteurization contamination patterns in fluid milk

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



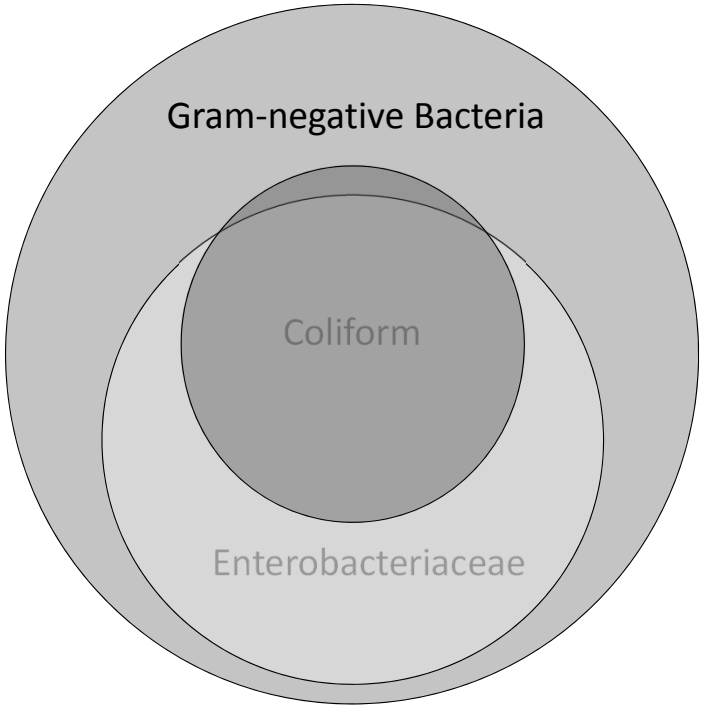
Post-pasteurization contamination patterns in fluid milk

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

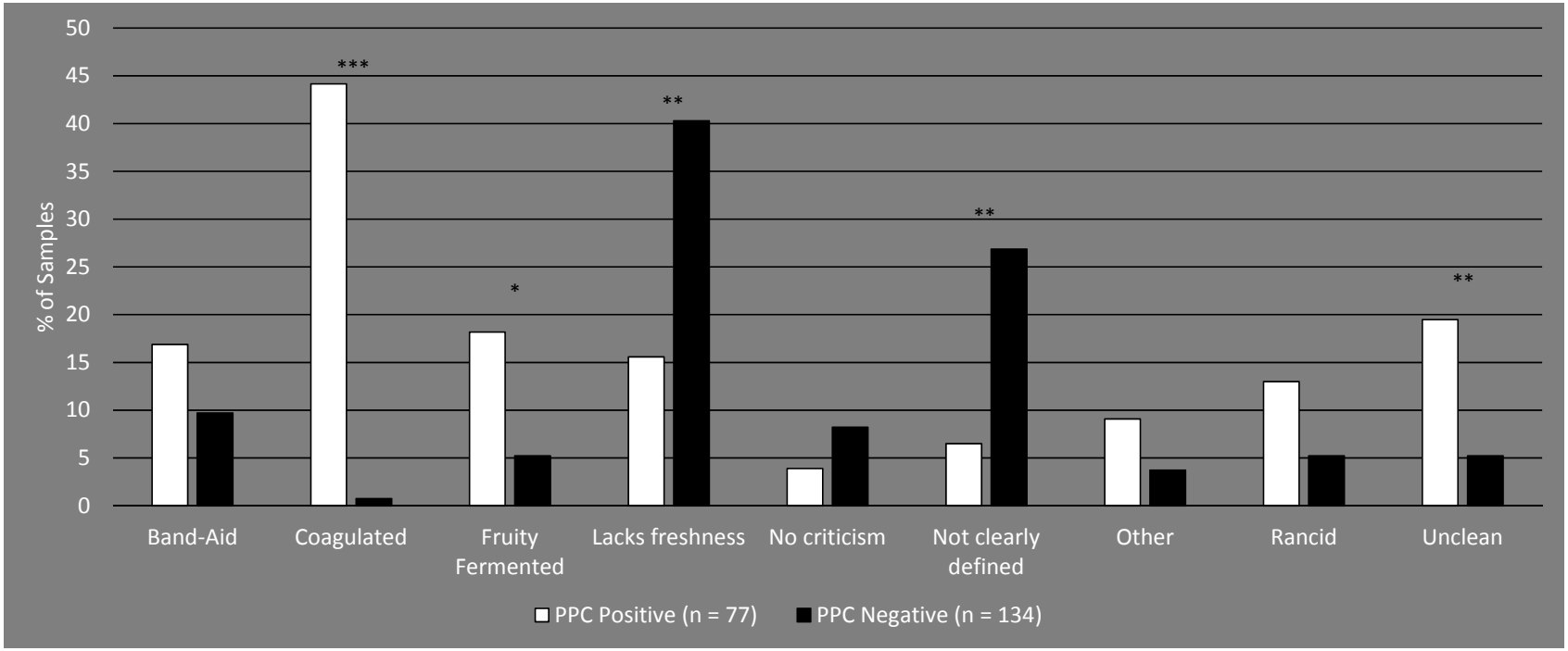


Post-pasteurization contamination patterns in fluid milk

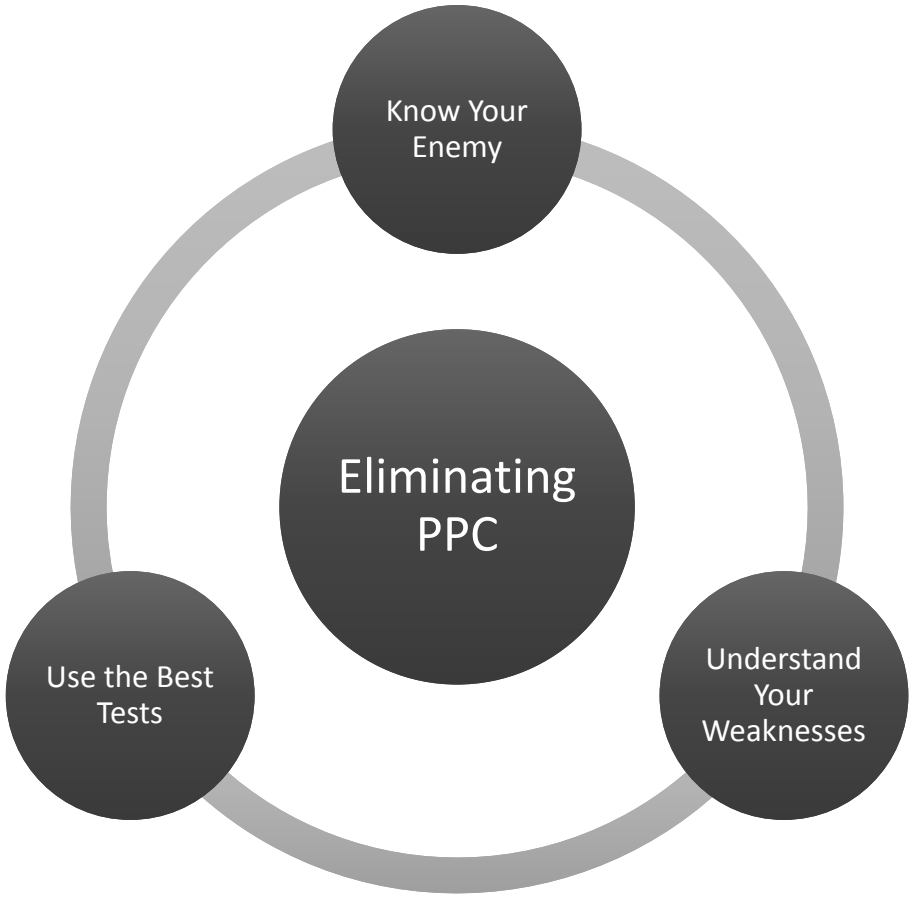
Group	Proportion of PPC Samples
Coliform	20%
Non-Coliform EB	7%
Non-Coliform, Non-EB Gram Negatives	100%

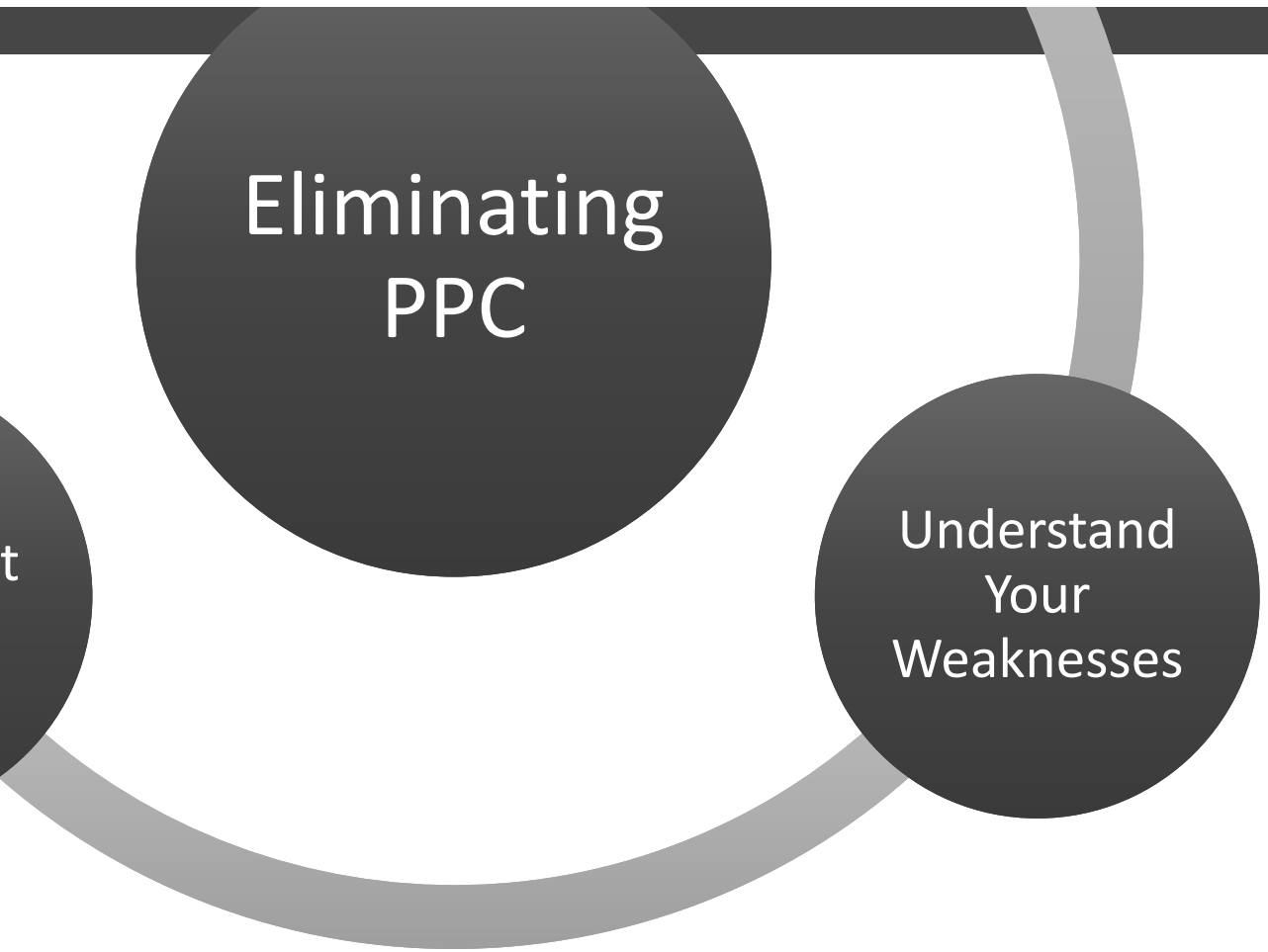


Post-pasteurization contamination – a barrier to high quality fluid milk

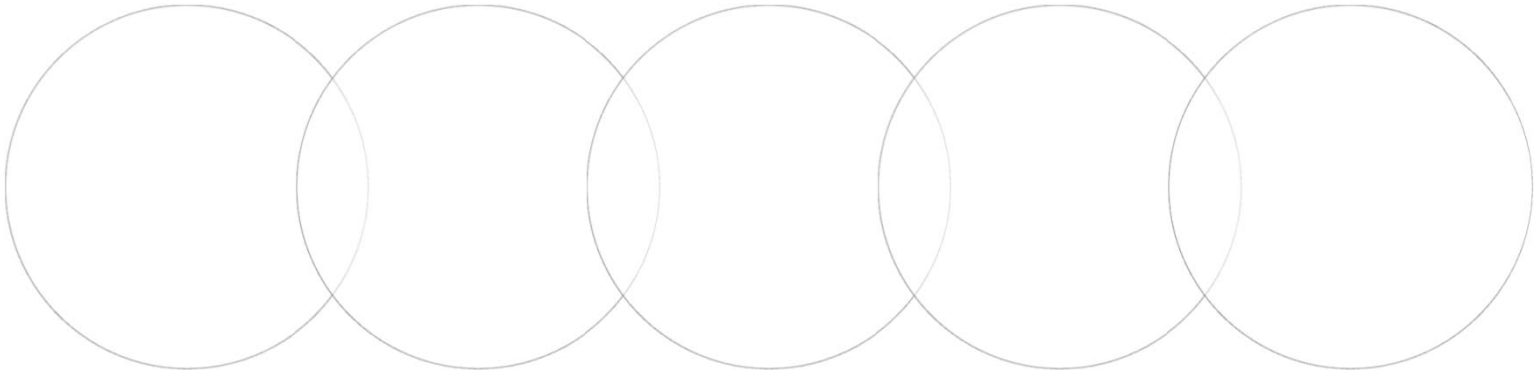


Reichler et al., unpublished


Know Your Enemy
Eliminating PPC
Use the Best Tests
Understand Your Weaknesses


Eliminating PPC
Understand Your Weaknesses
t

Understanding Your Weaknesses



Equipment Design

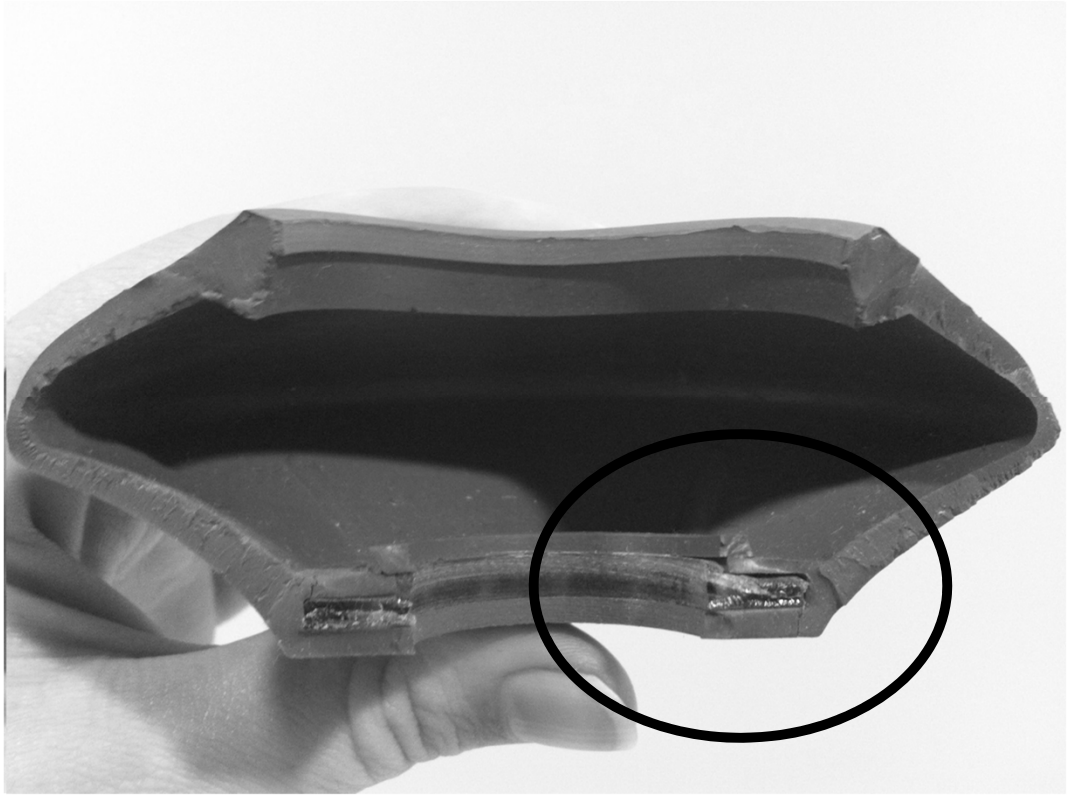
1. Cleanability
2. Construction Materials
3. Accessibility
4. No Liquid Collection
5. Hermetic Sealing
6. No Niches
7. Operational Performance
8. Maintenance Enclosures
9. Hygienic Compatibility
10. Cleaning Validation

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

	Date Processed	2015-08-03								2015-10-26								2016-02-08								2016-02-09	2016-05-20	2016-05-23					2016-05-24	2016-05-24	2016-05-24	
	Size	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	Gal	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	1/2 Pint	
	Container	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Plastic	Plastic	Paper	
	Filler	FED-2	FED-2	FED-2	FOGG-4	FOGG-4	N8-1	N8-2	N8-2	FED-2	FED-2	FED-2	FOGG-4	FOGG-4	N8-2	N8-2	N8-2	Fed-1	Fed-2	Fed-2	Fed-2	Fogg-4	Fogg-4	N8-2	N8-2	N8-1	Fed-2	Fogg-4	Fogg-4	Fogg-4	N8-2	N8-2	Fed-2	Fed-2	N8-2	
Repeat Isolation	Milk Type	2%	Skim	LF Choc	2%	LF Choc	LF Choc	2%	Skim	2%	Skim	LF Choc	2%	LF Choc	2%	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	Skim	2%	NF Choc	Skim	LF Choc	2%	Skim	Choc	2%	Skim	2%	Skim	LF Choc	**Genus**
	3																												X							*Limnohabitans*
•	9														X																			X		*Pseudomonas*
•	11					X																											X			*Pseudomonas*
•	13				X	X					X				X	X							X	X		X	X	X		X						*Pseudomonas*
•	14																						X				X						X			*Pseudomonas*
•	16		X			X		X							X	X							X			X		X	X		X	X				*Pseudomonas*
	17																						X													*Lelliottia*
•	18			X																						X										*Raoultella*
•	23									X						X		X								X						X				*Pseudomonas*
	25			X		X	X																													*Pantoea*
	26			X																																*Pseudomonas*
	27			X																																*Pantoea*
	28			X																																*Kluyvera*
	29			X																																*Raoultella*
	30					X																														*Psychrobacter*
	31					X																														*Acinetobacter*
	32					X																														*Klebsiella*
	36																									X										*Ewingella*
•	41																	X																	X	*Stenotrophomonas*
	51																							X												*Pseudomonas*
	52																									X										*Pseudomonas*
	54																					X														*Curvibacter*
	56																							X		X										*Rahnella*
	60														X																					*Pseudomonas*
	61														X																					*Serratia*

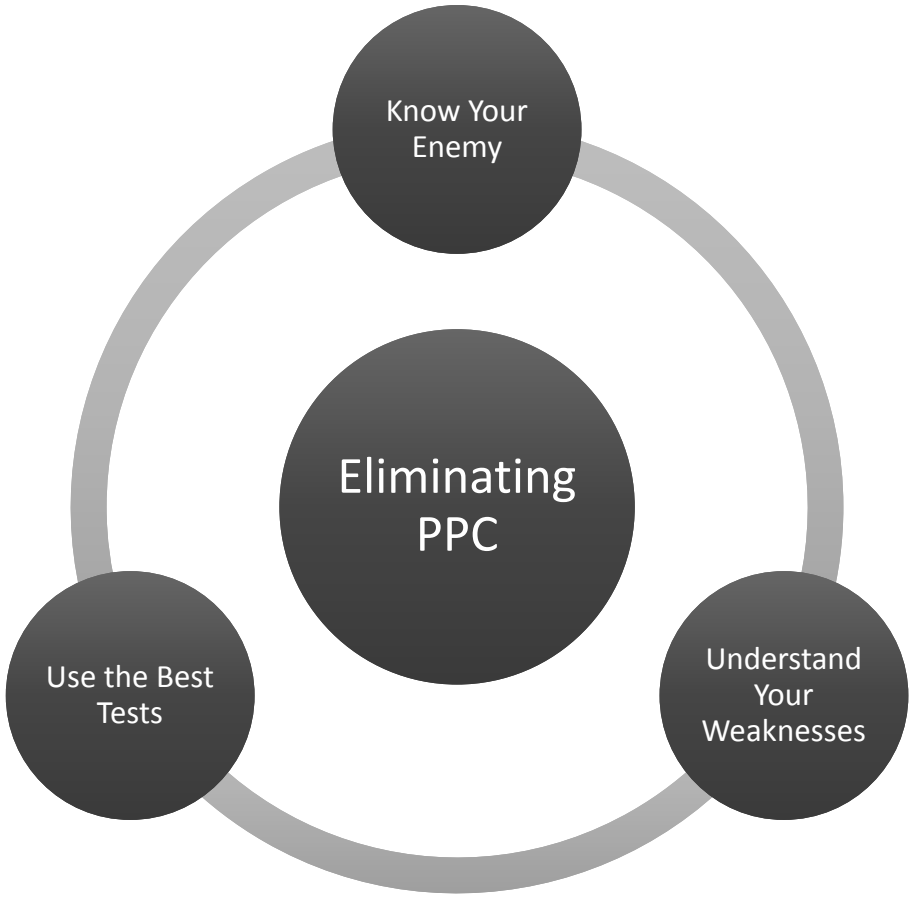
Repeat Isolation	Date Processed	2015-08-03								2015-10-26								2016-02-08								2016-02-09	2016-05-20	2016-05-23					2016-05-24	2016-05-24	2016-05-24	
	Size	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	Gal	1/2 Gal	1/2 Gal	1/2 Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	Gal	Gal	Gal	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	1/2 Pint	
	Container	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Paper	Plastic	Plastic	Plastic	Plastic	Paper	Paper	Plastic	Plastic	Paper	
	Filler	FED-2	FED-2	FED-2	FOGG-4	FOGG-4	N8-1	N8-2	N8-2	FED-2	FED-2	FED-2	FOGG-4	FOGG-4	N8-2	N8-2	N8-2	Fed-1	Fed-2	Fed-2	Fed-2	Fogg-4	Fogg-4	N8-2	N8-2	N8-1	Fed-2	Fogg-4	Fogg-4	Fogg-4	N8-2	N8-2	Fed-2	Fed-2	N8-2	
	Milk Type	2%	Skim	LF Choc	2%	LF Choc	LF Choc	2%	Skim	2%	Skim	LF Choc	2%	LF Choc	2%	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	Skim	2%	NF Choc	Skim	LF Choc	2%	Skim	Choc	2%	Skim	2%	Skim	LF Choc	**Genus**
	3																												X							*Limnohabitans*
•	9														X																			X		*Pseudomonas*
•	11					X																											X			*Pseudomonas*
•	**13**				X	X					X				X	X							X	X		X	X	X		X						***Pseudomonas***
•	14																						X				X						X			*Pseudomonas*
•	16		X			X		X							X	X							X			X		X	X		X	X				*Pseudomonas*
	17																						X													*Lelliottia*
•	18			X																						X										*Raoultella*
•	23									X						X		X								X						X				*Pseudomonas*
	25			X		X	X																													*Pantoea*
	26			X																																*Pseudomonas*
	27			X																																*Pantoea*
	28			X																																*Kluyvera*
	29			X																																*Raoultella*
	30					X																														*Psychrobacter*
	31					X																														*Acinetobacter*
	32					X																														*Klebsiella*
	36																									X										*Ewingella*
•	41																	X																	X	*Stenotrophomonas*
	51																							X												*Pseudomonas*
	52																									X										*Pseudomonas*
	54																					X														*Curvibacter*
	56																							X		X										*Rahnella*
	60														X																					*Pseudomonas*
	61														X																					*Serratia*

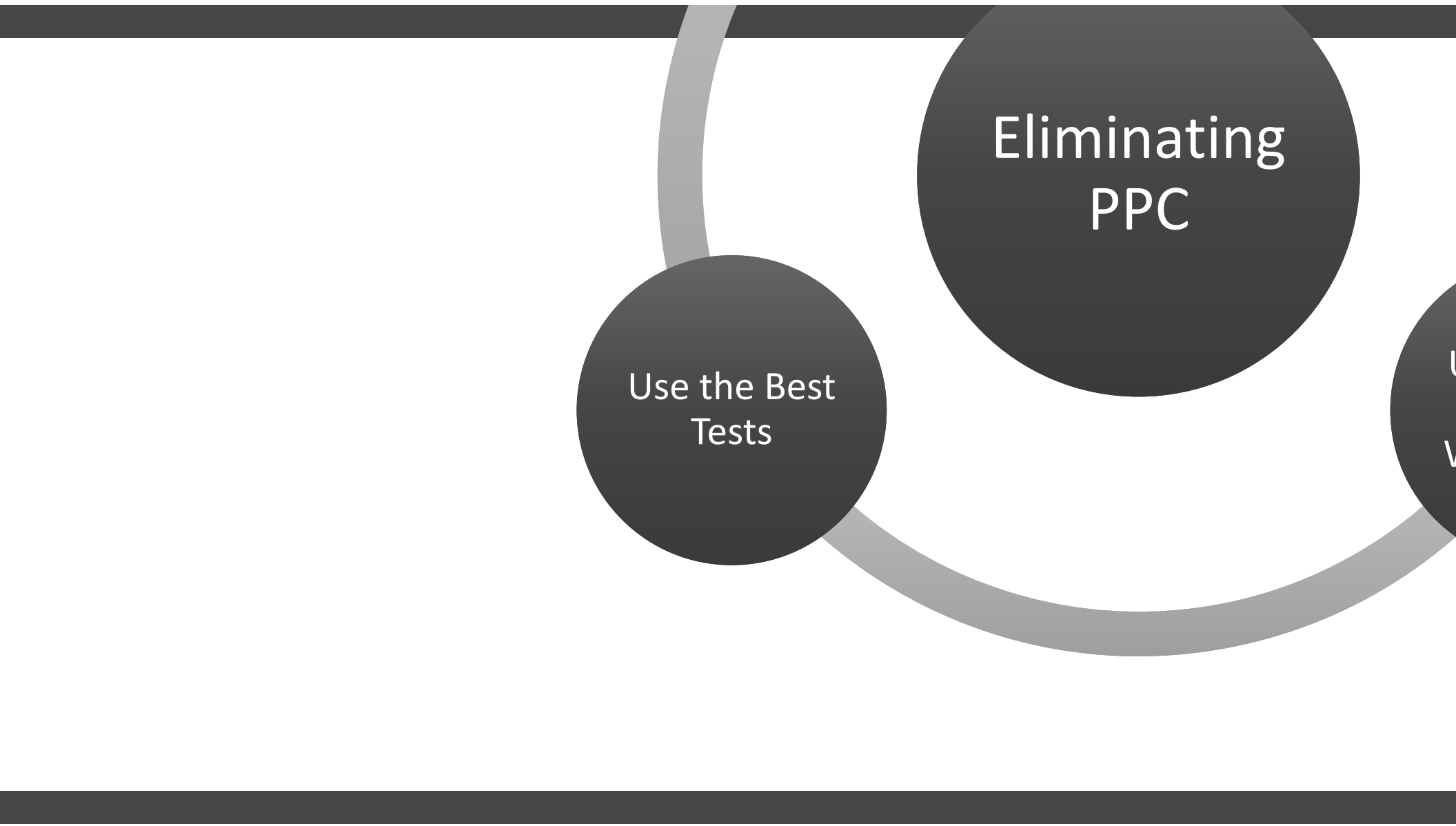
Repeat Isolation	Date Processed	2015-08-11						2015-10-27						2016-02-09						2016-05-24						
	Size	1/2 Gal	1/2 Gal	qt	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	qt	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	qt	1/2 Pint	1/2 Pint	1/2 Pint	1/2 Gal	1/2 Gal	qt	1/2 Pint	1/2 Pint	1/2 Pint	
	Container	Plastic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

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

Repeat Isolation																																					
	Date Processed	2015-08-03								2015-10-05									2016-02-08								2016-04-18								
	Size	1/2 Pint	1/2 Gal	1/2 Gal	1/2 Gal	pt	Gal	Gal	Gal	1/2 Gal	qt	qt	pt	pt	pt	Gal	Gal	Gal	qt	qt	1/2 Gal	pt	pt	Gal	Gal	Gal	1/2 Gal	1/2 Gal	1/2 Gal	pt	pt	pt	Gal	Gal	Gal
	Container	Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic
	Filler	N8	FED-2	FED-2	FED-2	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	PET	Fogg	Fogg	Fogg
	Milk Type	2%	2%	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	2%	Skim	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	Skim	2%	Skim	LF Choc	2%	Skim	LF Choc	2%	2%	Skim	Skim	LF Choc	Choc	2%	Skim	LF Choc	Genus
	1																			X																*Acinetobacter*
•	2			X								X																								*Acinetobacter*
	8			X																																*Pseudomonas*
	9																X																			*Pseudomonas*
	11																						X													*Pseudomonas*
•	16	X	X	X																			X													*Pseudomonas*
	22									X								X																		*Pseudomonas*
	24		X					X																												*Pseudomonas*
	41																															X				*Stenotrophomonas*
	67																														X					*Pseudomonas*
	84																		X																	*Obesumbacterium/Ewingella*
	85																			X			X		X											*Pseudomonas*
	86																			X																*Klebsiella*
	87																						X													*Pseudomonas*
	88																						X													*Pseudomonas*
	106																													X						*Pseudomonas*
	112																																	X		*Pseudomonas*
	113																														X					*Citrobacter*

Date Processed		2015-08-03								2015-10-05									2016-02-08								2016-04-18									
Size		1/2 Pint	1/2 Gal	1/2 Gal	1/2 Gal	pt	Gal	Gal	Gal	1/2 Gal	qt	qt	pt	pt	pt	Gal	Gal	Gal	qt	qt	1/2 Gal	pt	pt	Gal	Gal	Gal	1/2 Gal	1/2 Gal	1/2 Gal	pt	pt	pt	Gal	Gal	Gal	
Container		Paper	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	Plastic	
Filler		N8	FED-2	FED-2	FED-2	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	Fogg	Fogg	Fogg	FED-2	FED-2	FED-2	PET	PET	PET	Fogg	Fogg	Fogg	
Repeat Isolation	Milk Type	2%	2%	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	2%	Skim	Skim	LF Choc	LF Choc	2%	Skim	LF Choc	2%	Skim	2%	Skim	LF Choc	2%	Skim	LF Choc	2%	2%	Skim	Skim	LF Choc	Choc	2%	Skim	LF Choc	Genus
	1																			X																*Acinetobacter*
•	2			X								X																								*Acinetobacter*
	8			X																																*Pseudomonas*
	9																X																			*Pseudomonas*
	11																						X													*Pseudomonas*
•	16	X	X	X																			X													*Pseudomonas*
	22									X								X																		*Pseudomonas*
	24		X					X																												*Pseudomonas*
	41																															X				*Stenotrophomonas*
	67																														X					*Pseudomonas*
	84																		X																	*Obesumbacterium/Ewingella*
	85																			**X**			**X**		**X**											***Pseudomonas***
	86																			X																*Klebsiella*
	87																						X													*Pseudomonas*
	88																						X													*Pseudomonas*
	106																													X						*Pseudomonas*
	112																																	X		*Pseudomonas*
	113																														X					*Citrobacter*


Know Your Enemy
Eliminating PPC
Use the Best Tests
Understand Your Weaknesses


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

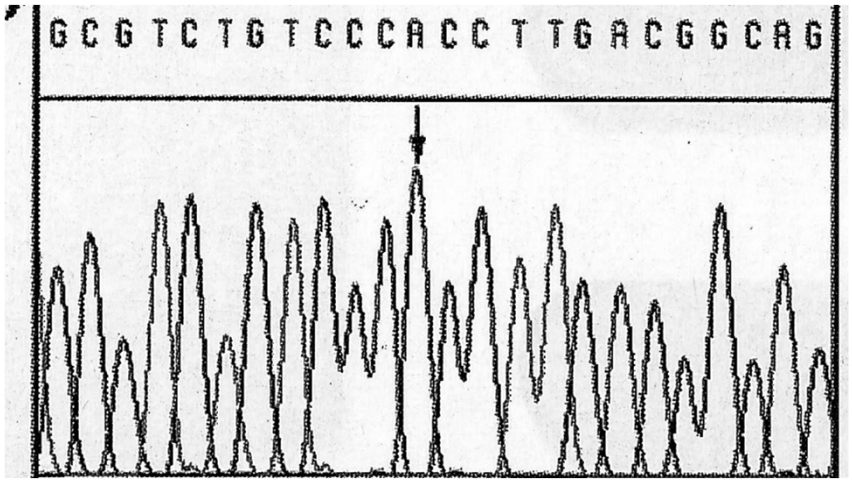
Media	Pre-Incubation Temperature (°C)	Sensitivity (%)	Specificity (%)
Crystal Violet Tetrazolium (CVTA) Agar	21	70	93
Enterobacteriaceae (EB) Petrifilm	21	23	100
Coliform Petrifilm	21	15	100
Crystal Violet Tetrazolium (CVTA) Agar	13	33	98
Enterobacteriaceae (EB) Petrifilm	13	7	100
Coliform Petrifilm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

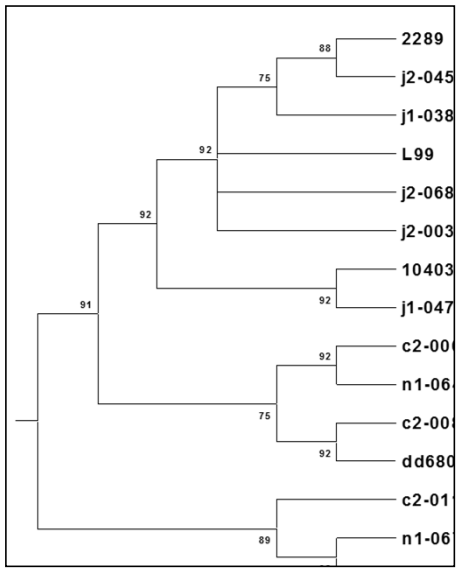
Alles et al., submitted

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)

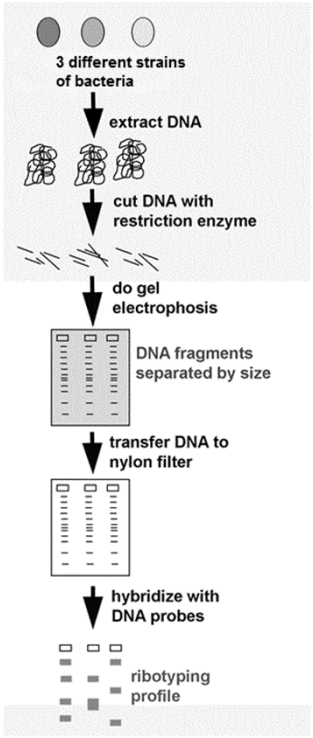
DNA sequencing-based subtyping

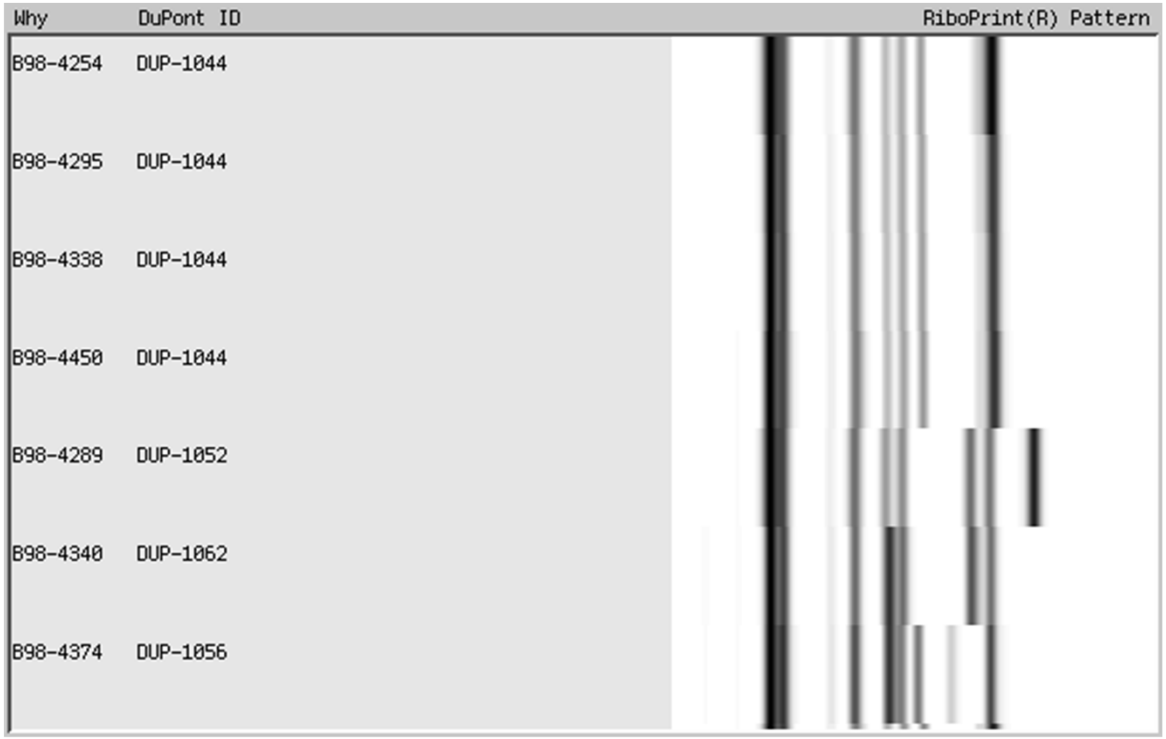





```
Isolate 1 AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 2 AACATGCAGACTGACGATTCGTCGTAGGCTAGACGTTGACTG
Isolate 3 AACATGCAGACTGACGATTCGACGTAGGCTAGACGTTGACTG
Isolate 4 AACATGCATACTGACGATTCGACGAAGGCTAGACGTTGACTG
```

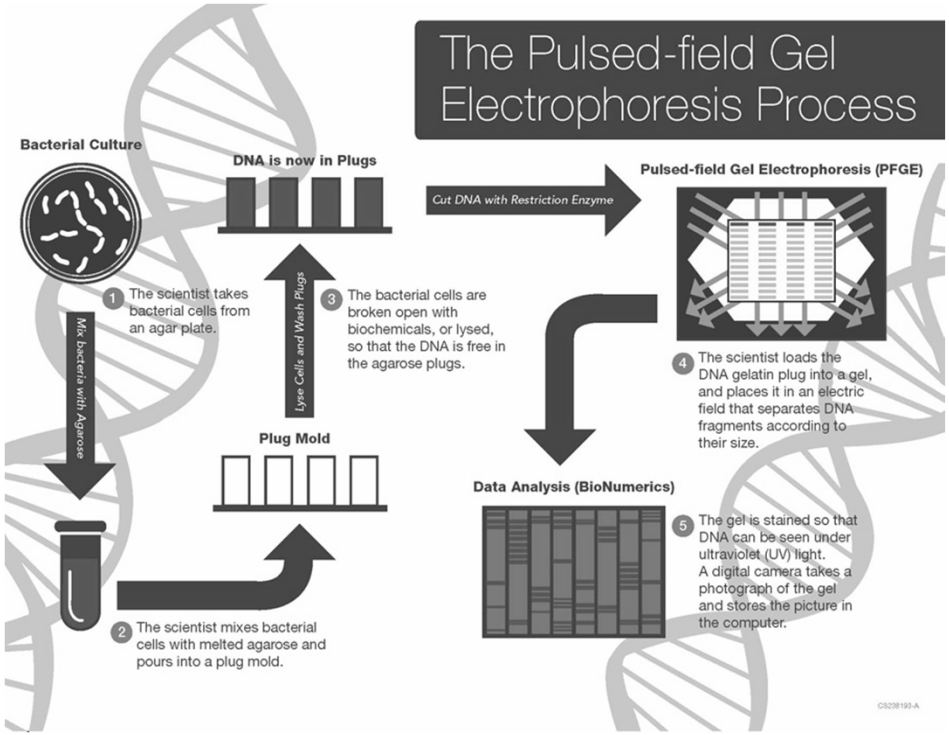
Ribotyping

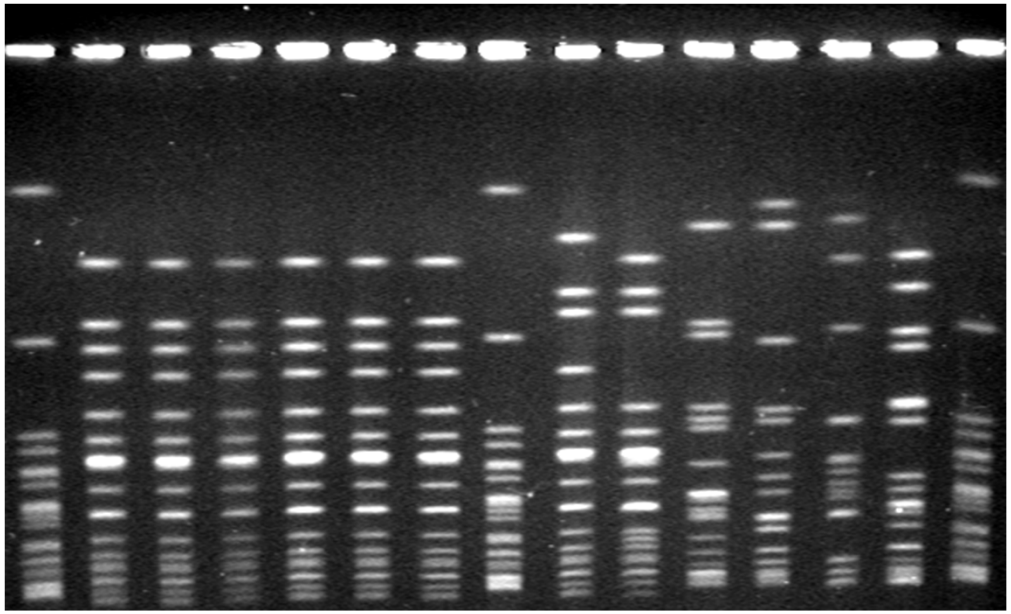




https://courses.cit.cornell.edu/courses/biomi290/microscopycases/methods/ribotype.htm

Pulsed Field Gel Electrophoresis (PFGE)





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

Whole Genome Sequencing (WGS) for improved subtyping

AEM Accepted Manuscript Posted Online 26 June 2015
Appl. Environ. Microbiol. doi:10.1128/AEM.01049-15
Copyright © 2015, American Society for Microbiology. All Rights Reserved.

RESEARCH

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

Henk C. den Bakker, Marc W. Allard, Dianna Bopp, Eric W. Brown, John Fontana, Zamin Iqbal, Aristea Kinney, Ronald Limberger, Kimberlee A. Musser, Matthew Shudt, Errol Strain, Martin Wiedmann, and William J. Wolfgang

7 *Author Affiliation:* [1]Department of Food Science, College of Agriculture and Life Sciences.

8 Cornell University, Ithaca, NY 14853.

A Case Study: When Cheese Gets the Blues

- Low salt, low acid cheese (pH~5.9-6.0) 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.[1]	Isolate ID[2]	Isolate origin (sampling)[3]	Color development on PDA[4]	Fluorescence under UV[5]	Cheese inoculation[6]	Isolate similarity for selected tests[7]	
						PFGE	Ribotype
W5–203	*Pseudomonas fluorescens*	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5–206	*P. fluorescens*	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5–207	*P. fluorescens*	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5–208	*P. fluorescens*	PT-1 cheese curd (1)	Blue	Yes	Blue	1	1
W5–287	*P. fluorescens*	PT-2 cheese curd (1)	Blue	Yes	Blue	1	1
W5–296	*P. fluorescens*	PT-2 cheese curd (1)	Blue	Yes	Blue	2	1
W5–319	*P. fluorescens*	CV-2 agitator drain tube swab (3)	No color	No	No color	3	1
W5–325	*P. fluorescens*	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5–326	*P. fluorescens*	CV-1 agitator track swab (3)	Blue	Yes	Blue	1	1
W5–327	*P. fluorescens*	CV-1 agitator track swab (3)	No color	No	No color	3	1
R5–199*	*P. fluorescens*	Dairy plant isolate	No color	No	No color	NA	1
R5–202*	*P. fluorescens*	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