Heat-Resistant Psychrotrophs (HRP) and Their Effect on Market Milk Quality

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CHANGES IN DAIRY INDUSTRY

- Improved Sanitation
- Improved Equipment
- Increased Regulatory Involvement

- Effective industry quality efforts resulted in effective control of post pasteurization contamination (PPC).

2

Control of PPC improved:

- Shelf life
- Safety
- Quality
- Fluid milk shelf-life has improved from 10-14 days to 18-21 days
- Heat-resistant psychrotrophic (HRP) bacteria are the barrier to improving beyond 18 – 21 days.

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Challenge for Dairy Industry

- Recognize the effect of heat-resistant psychrotrophic bacteria on market milk
- Establish methods of monitoring and controlling heat-resistant psychrotrophic bacteria

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State of the Dairy Industry

- The need to recognize the effect of HRP bacteria on market milk

  Not a new condition. 1970s:

  - Floyd Bodyfelt, Univ. of Oregon
  - E. M. Mikolajcik, Ohio State
- Extend pull dates
- Need to establish & implement methods of monitoring and controlling HRP.

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MONITORING MICROBIAL CONTAMINATION OF DAIRY PRODUCTS

Key Points .

- There are many sources and mechanisms of microbial contamination (MC).
- Psychrotrophic contamination (PC) normally is quite low.
- Testing must detect very low levels of PC.
- When PC determines shelf life, the growth rate is the primary factor affecting quality.
- Continuous monitoring is needed for a successful quality control program.

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PSYCHROTROPHIC SPOILAGE
Pasteurized Milk

Bacterial Group	Common Source	Common Defect
Gram Negative Rods	Post-Pasteurization Contamination	Bitter, Fruity, Putrid, Unclean, Ropiness
Gram Positive Cocci	Thermoduric and/or Environmental Contamination	Acid Souring, Ropiness
Gram Positive Rods	Thermoduric	Sweet Curdling, Bitter .

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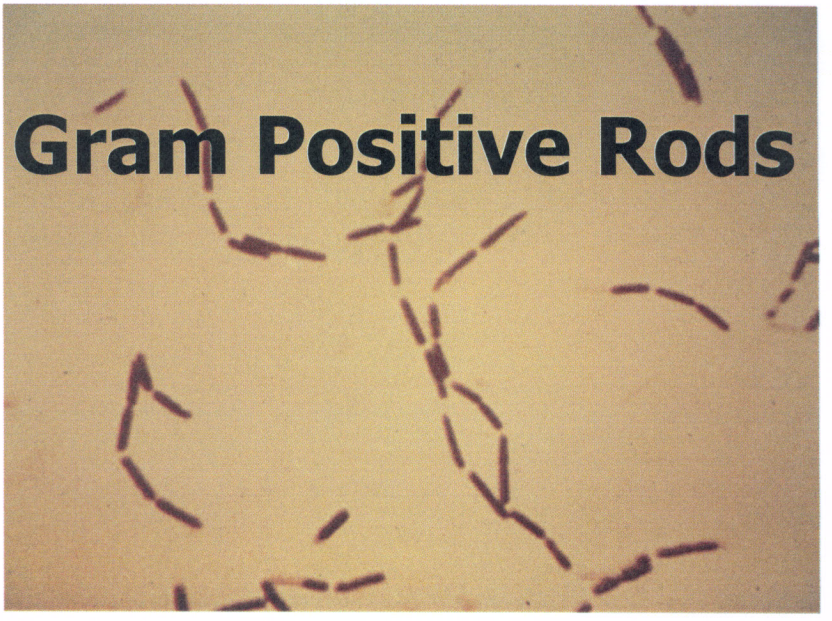

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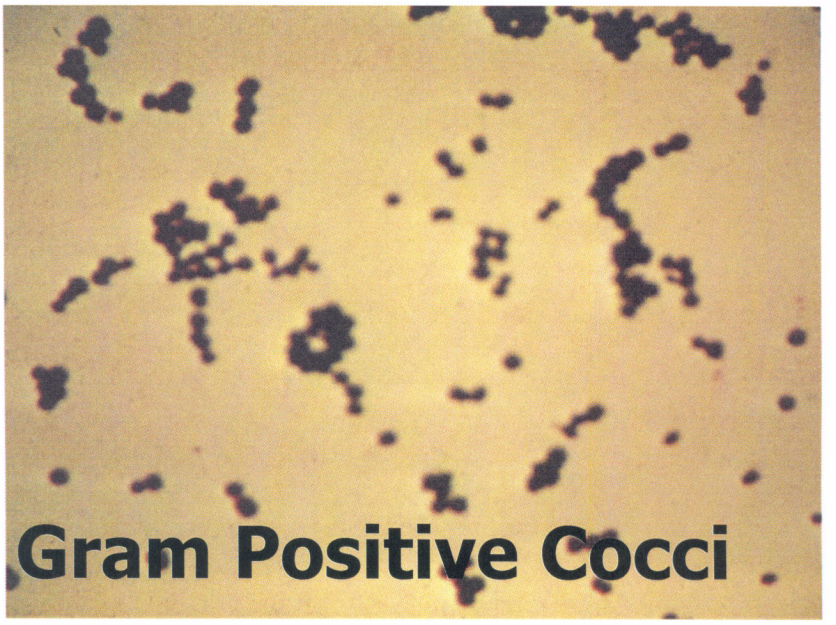

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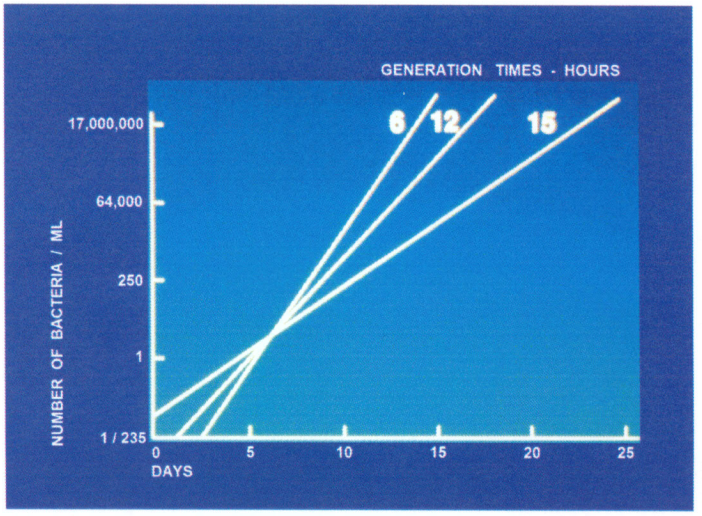

Time to reach a count of 17,000,000 / ml with generation times of 6, 12, and 15 hr and a count of 1 / ml after 5 days of storage.
Marshall, R.T. and R. Appel, 1973. Sanitary conditions in twelve fluid milk processing plants as determined by use of the rinse filler method. J. of Milk and Food Technol., 38(4) 230.

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RELATIONSHIPS AMONG CONTAMINATION RATE, BACTERIAL GROWTH RATE AND PRODUCT SPOILAGE

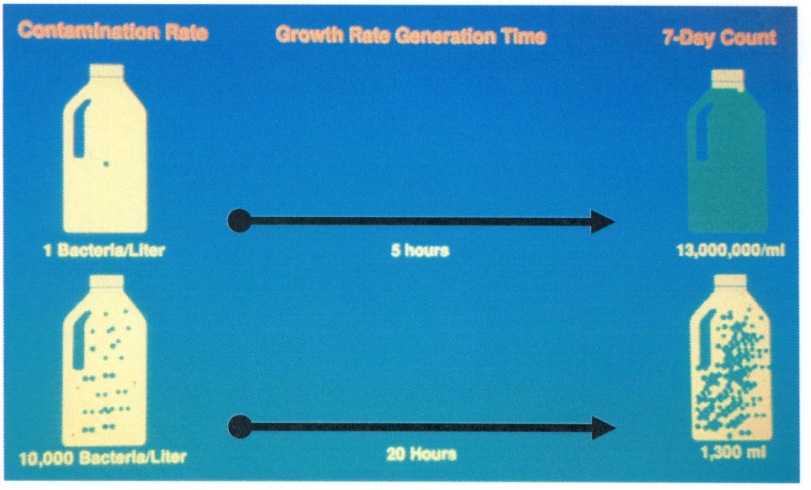

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Table 1. Growth parameters for *Bacillus spp.* grown in milk.

Species	Temperature of Growth			
	6°C		10°C	
	Lag [a]	Gen [b]	Lag	Gen
B. Cereus [c]	78	17	24	4
B. Circulans [d]	33	11	31	7
B. Mycoides [e]	92	22	33	4

[a] **Lag time in h.**
[b] **Generation time in h.**
[c] **Mean of four strains.**
[d] **Mean of four strains.**
[e] **Mean of two**

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University of Minnesota Study

To determine the effect of psychrotrophic spores on today's milk quality, plate counts were conducted on samples collected with the QMI® Aseptic Sampler and the QMI® Composite Bag from the discharge of the HTST.

- Samples were free from gram-negative bacteria
- Gram-positive (psychrotrophic sporeforming) bacteria grew in some samples.

The study showed that psychrotrophic spores can affect the quality of market milk.

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University of Minnesota Study

Effect of Gram-Positive Psychrotrophic Bacteria on Dairy Product Quality

Sample	Dairy	Bag	Week 3	Week 4
			CFU/ml	CFU/ml
			Gram +	Gram +
3/16/2004	Plant B	A	<10	<10
Tuesday		B	<10	<10
3/17/2004	Plant A	A	7.56×10^4	1.0×10^6
Wednesday		B	2.33×10^4	1.93×10^6
3/24/2004	Plant A	A	1.41×10^6	1.05×10^7
Wednesday		B	0.95×10^6	3.1×10^7
3/31/2004	Plant A	A	<10	<10
Wednesday		B	<10	<10
4/2/2004	Plant B	A	6.7×10^5	2.5×10^6
Friday		B	4.96×10^5	6.5×10^6
4/15/2004	Plant B	A	<10	6 x 10
Thursday		B	<10	<10

Weekly Counts from Milk Samples Taken at the HTST and stored at 45° F

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Cornell University Study

Found that the most significant factor affecting shelf life was the growth of heat resistant, psychrotrophic, gram-positive bacteria.

- 50% of samples: bacteria counts were greater than 1,000,000 CFU/mL after 17 days of refrigerated storage
- 87% of microbial colonies analyzed were gram-positive rods
- *Paenibacillus, Bacillus, Microbacterium*
- *Paenibacillus* appears to be gram-negative in staining procedures, but actually is gram-variable.

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Table 1. Psychrotrophic bacteria in pasteurized milk samples (1 of 2)

Day of sampling	Time of sampling	Total psychrotrophs (Mean Log CFU / mL) Initial	Final
Mon	1	<1	6.56
	2	<1	6.54
	3	<1	6.80
	4	<1	6.61
Tues	1	<1	5.85
	2	<1	6.55
	3	<1	6.95
	4	<1	6.39
Wed	1	<1	5.77
	2	<1	6.42
	3	<1	5.88
	4	<1	6.82

Initial = day 0
Final = after 18 days incubation at 7°C
NA = Not analyzed (missing / ruptured package in shipment)

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Table 1. Psychrotrophic bacteria in pasteurized milk samples (2 of 2)

Day of sampling	Time of sampling	Total psychrotrophs (Mean Log CFU / mL) Initial	Final
(Continued from previous slide)			
Thurs	1	<1	6.73
	2	<1	6.70
	3	<1	6.79
	4	NA	NA
Fri	1	<1	6.16
	2	NA	NA
	3	<1	6.14
	4	<1	6.91

Initial = day 0
Final = after 18 days incubation at 7°C
NA = Not analyzed (missing / ruptured package in shipment)

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Why Spore Forming Bacteria are a Problem to the Dairy Industry

- Spores are resistant to:
  - Chemicals
  - Heat
  - Sanitizers
- Raw milk dairy equipment may be selecting for psychrotrophic spore-forming bacteria
- HTST - Heat shock spores into out growth

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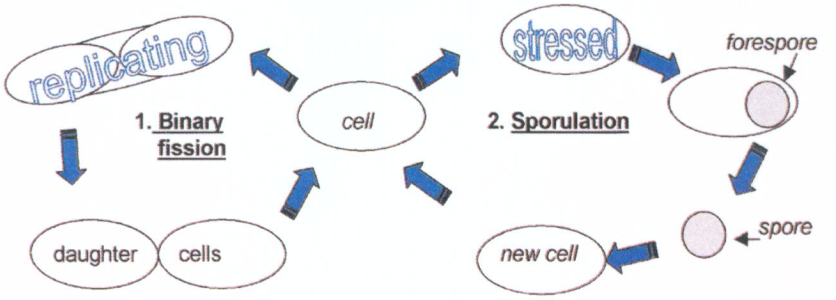
SPORULATION OF GRAM POSITIVE BACTERIA



Raw milk dairy equipment creates sporulation conditions

HTST – heat shocks spores into outgrowth

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RECOMMENDED INDUSTRY SPECIFICATIONS FOR PASTEURIZED MILK

Initial Evaluation

SPC	500 – 1000 / ml
Coliform	Any level requires immediate correction
Sensory	No detectable defects

Moseley Keeping Quality Evaluation - At ½ of Code:

SPC	2000 – 20,000/ml
Coliform	Any level requires immediate correction
Sensory	No detectable defects

At End of Code:

SPC	< 1,000,000/ml
Coliform	Any level requires immediate correction
Sensory	No detectable defects .

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TRADITIONAL LABORATORY TESTS FOR PASTEURIZED FLUID MILK

- SPC
- Coliform
- Moseley shelf-life test
- Sensory evaluation
- Line sampling

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TRADITIONAL LABORATORY TESTS FOR RAW MILK

- SPC
- LPC
- Coliform
- PI
- SCC
- Inhibitors / Antibiotics

Note: these tests do not correlate with HRP or the shelf-life of pasteurized fluid milk

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Mansel Griffiths, Ph.D. University of Guelph

- Research of heat-resistant psychrotrophic populations:
  - 19 per liter in poor quality milk
  - 15 per liter in good quality milk

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RECOMMENDED ADDITIONAL LABORATORY METHODS FOR THE FLUID MILK INDUSTRY

- Stress Test
- Packaging analysis
- Determining bacterial types
- Environmental testing
  - Compressed Air
  - Glycol
  - Chill water
  - Water
- Screening raw milk
  - Modified Mikolajcik Method
  - QMI® Heat-Resistant Psychrotrophic Test

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Screening Raw Milk for Heat-Resistant Psychrotrophs (HRP)

- Modified Mikolajcik - Feijoo and Bodyfelt
- (100 ml raw milk in glass)
  - Heat shock 75C for 20 minutes
  - Store sample 8 to 10 days at 45F / 7C
  - Plate on standard methods agar

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QMI® Heat-Resistant Psychrotrophs (HRP) Test

- Aseptically collect a sample of raw milk
  using the 250ml QMI Sampling Bag and
  the QMI Aseptic Sampler,
- Lab Pasteurize the sample in the bag
  at 75° C for 20 minutes,
- Place in a 45° F incubator, and
- Determine Standard Plate Counts at the
  end of code. Identify bacteria using
  gram-staining procedures.

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Problems created by Heat-Resistant Psychrotrophs (HRP)

- Out of spec at end of code
- Consumer complaints
- Reduced quality:
  physical and sensory defects

Research validates these issues

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Table 4. Germination and outgrowth of spore-formers at 7°C and 11°C

Time (Days)	250 ml	
	7°C	11°C
Full-fat milk		
3	-	2.6
5	-	5.5
7	2.9	6.9
10	5.0	7.5
14	6.0	6.8

Results are geometric means for 12 milks.

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Post-Pasteurization Contamination vs Heat Resistant Psychrotrophs

Post-Pasteurization Contamination:

1. Spoilage 10-14 days
2. Gram-Negative Bacteria (e.g. *Pseudomonas*)
3. Stress Test: growth
4. Mosely Test: out of specifications
5. Line Sample: gram-negative
6. Requires dissolved oxygen for optimal growth

Heat-Resistant Psychrotrophs:

1. Spoilage 18+ days
2. Gram-Positive Bacteria
3. Stress Test: no-growth
4. Mosely Test: within specifications
5. Line Sample: gram-positive
6. Does not require dissolved oxygen for optimal growth

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Monitoring Raw Milk For HRP

- Sample size

  Dr. Mansel Griffiths, U of Guelph, found average contamination rate is 17 HRP per liter
- Modified Mikolajcik Method
- QMI method

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Procedure One:

To determine if psychrotrophic sporeformers are affecting the quality of your market milk products:

Sampling

1. Use the QMI® Aseptic Sampling System, aseptically obtain a 2L or 5L pasteurized milk sample using the QMI® Composite Sampling Bag

continued ...

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Procedure One, continued:
To determine if psychrotrophic spore formers are affecting the quality of your market milk products:

Testing

2. Incubate the sample in the bag for 18 – 24 days (end of code) at 45°F (7°C).
3. Conduct a Standard Plate Count.
4. Identify any bacteria using gram stain procedures or other procedures for samples with counts greater than 1,000,000/ml.

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Procedure Two:
QMI® Heat-Resistant Psychrotrophic (HRP) Test

Objective: Determine Sources

1. Aseptically fill a 250ml bag with raw milk
2. Lab pasteurize (LP) sample at 75°C for 20 minutes
3. Place in 45°F (7°C) keeping quality incubator
4. Determine SPC at 18 days or end of code
5. Identify bacteria

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Lab pasteurize QMI® 250ml aseptic sampling bag

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Table A: Growth of Pseudomonas in QMI Composite Bag vs. Syringe

	Day 7	Day 14	Day 21
Control Bag 2	<1	<1	<1
Bag 1	80	2.2×10^6	6.5×10^7
Bag 2	70	1.0×10^6	10.5×10^7
Bag 3	70	1.7×10^6	6.0×10^7
Control Syringe	<1	<1	<1
Syringe 1	<1	3	21.5
Syringe 2	<1	<1	3.5
Syringe 3	1	1	193

Inoculation rate was about 4 bacteria per 10 ml of sterile milk

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Pseudomonas huttiensis

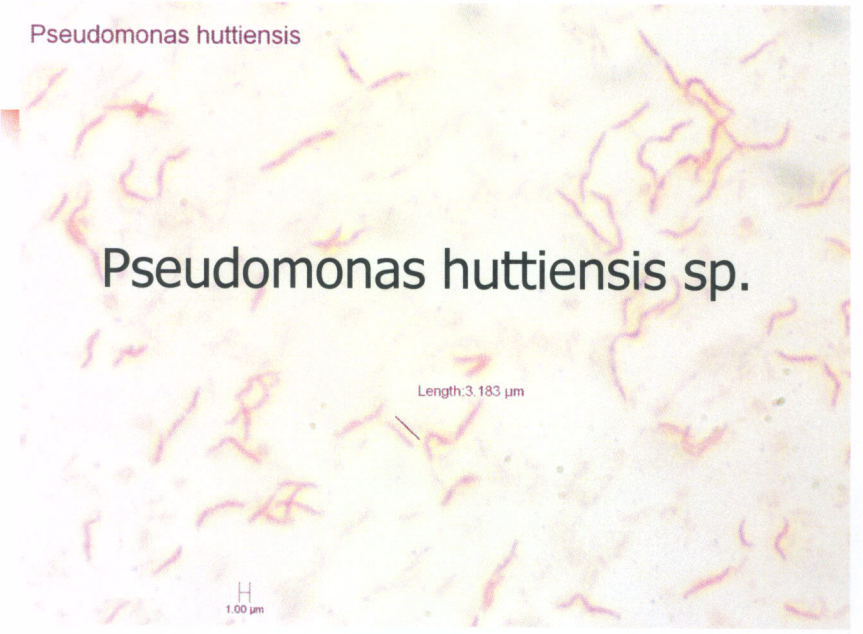

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Table B: Growth of *Paenibacillus sp* in QMI Composite Bag vs. Syringes

	Day 7	Day 14	Day 21	Day 28
Syringe	1.7	6.5×10^1	1.1×10^4	4.7×10^4
Control	<1	<1	<1	<1
Bag 1	<1	1.6×10^2	7.3×10^3	7.3×10^3
Bag 2	<1	20×10^2	5.9×10^6	2.4×10^7
Bag 3	<1	8.7×10^1	1.2×10^4	3.0×10^4
Control	<1	<1	<1	<1

Inoculation rate was about 1 bacteria per 10 ml of sterile milk

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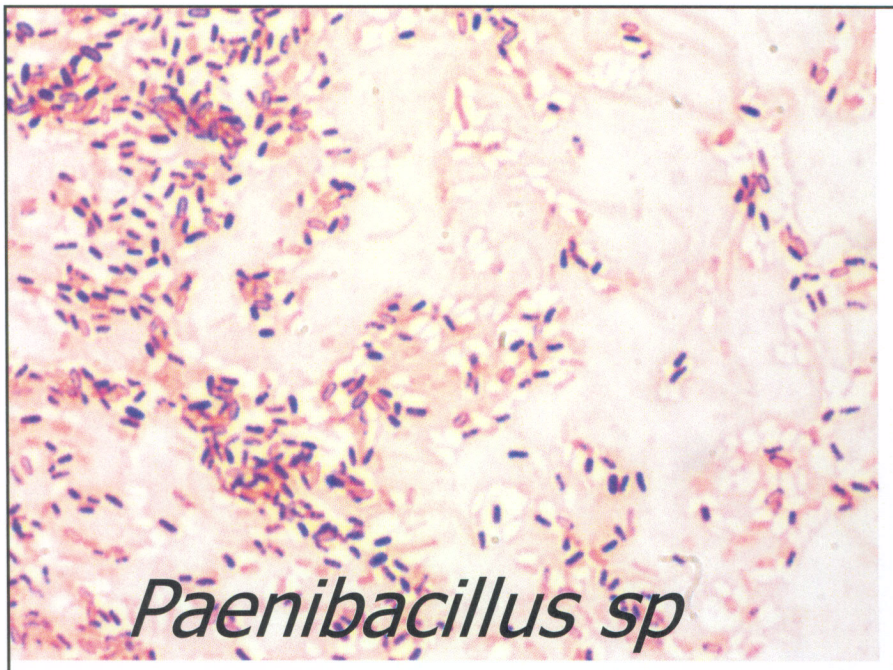

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Results for QMI
2 Liter Bag Samples from HTST

- Sample failure = product failure
- Gram + Bacteria

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Table 5. Germination and outgrowth of spores in milk incubated at 7°C or 11°C in bags with different OTR

	Days of incubation					
	3	5	7	10	14	OTR
7°C						
Bag 1	0	0	1.8	4.67	4.98	0.041
Bag 2	0	0	3.45	4.6	5.77	0.030
Bag 3	0	0	2.2	4.5	5.6	0.047
QMI bag	0	0	2.9	5.0	6.0	0.177
11°C						
Bag 1	0	0	5.7	5.8	6.7	0.041
Bag 2	0	0	5.7	6.7	6.6	0.030
Bag 3	0	0	5.7	6.7	6.7	0.047
QMI bag	2.6	5.5	6.9	7.5	6.8	0.177

Validation of assay using 11°C incubation
OTR = Oxygen Transmission Rates

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Raw Milk Handling Equipment: Source of Psychrotrophic Spores

- *Bacillus* species – tendency to form biofilms
- Cold environment of raw milk handing equipment - favors psychrotrophic bacteria
- Stresses to bacteria (i.e. removal of nutrients) can cause bacteria to sporulate

continued on next slide ...

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Raw Milk Handling Equipment: Source of Psychrotrophic Spores

continued ...

- Effective sanitation of raw milk handling equipment is often neglected
- High humidity conditions favor sporulation
- Contamination rates as low as 1/liter could cause quality defects in pasteurized milk

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QMI® Heat Resistant Psychrotrophic Test Results

		Sample A	Sample B
Plant Silos	1/23/2006	9.0×10^6	1.0×10^7
	1/29/2006	2.0×10^5	2.0×10^6
	2/02/2006	6.7×10^7	1.0×10^6
	2/03/2006	2.0×10^5	$< 10^4$
	2/08/2006	3.5×10^7	3.5×10^7
Farm Bulk Tanks	2/24/2006	$< 10^4$	$< 10^4$
	2/26/2006	3.0×10^5	$< 10^4$
	4/12/2006	2.7×10^6	5.0×10^5
	4/12/2006	$< 10^4$	$< 10^4$
LP samples stored at 45° F for 18 days			

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Corrective Action # 1

- Improve sanitation programs for raw milk handling equipment, particularly over-the-road tanker trucks
  - Sprayball monitoring
  - Visual inspection (dried surfaces)
  - CIP solutions (time, temp, concentration, pressure/flow)
  - Hygiene monitoring systems (e.g. ATP, etc.)
  - Routine acid washes

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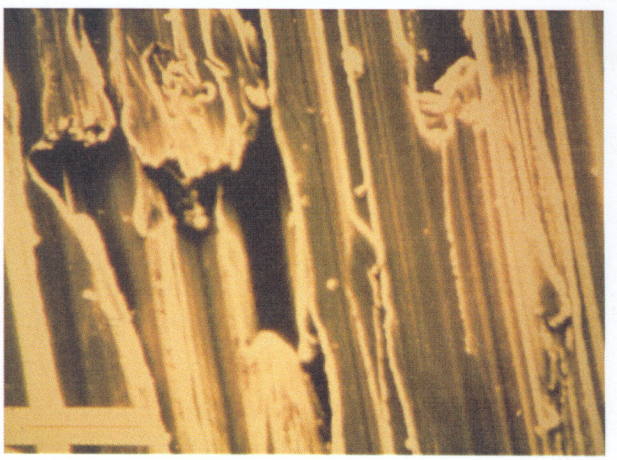
Polished Stainless Steel



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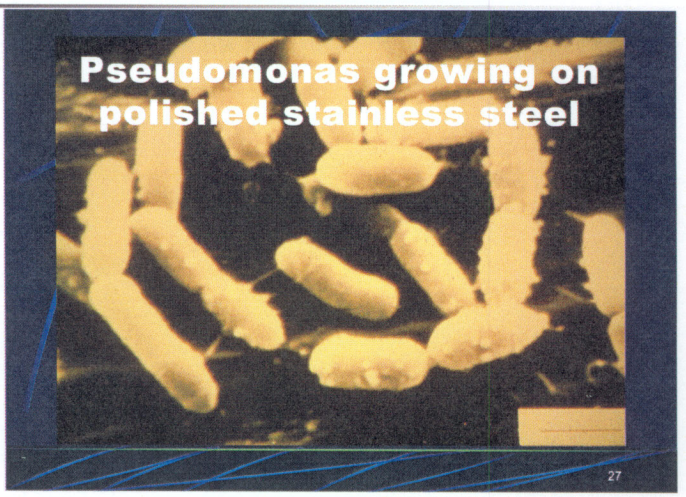
Polished Stainless Steel



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Corrective Action # 2

- Keep pasteurized products as cold as possible
  - HRP do not grow as well at temperatures below 40°F

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Corrective Action # 3

- Follow manufacturer's recommendations for de-sludging cycle and "shoot" times
- Sample HTST systems just before de-sludging

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Corrective Action # 4

- Educate, motivate and monitor raw milk suppliers.
- Educate, motive and monitor raw milk transport trucks.

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Conclusion

- Raw milk handling equipment requires the same level of care as pasteurized milk handling equipment
- Reduce the effect of HRP from raw milk by proactive quality management.

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Microbial Spoilage of Market Milk

Post-Pasteurization Contamination:	Heat-Resistant Psychrotrophs:
Spoilage 10-14 days	Spoilage 18+ days
Gram-Negative Bacteria	Gram-Positive Bacteria
Stress Test: growth	Stress Test: no-growth
Mosely Test: out of specifications	Mosely Test: within specifications
ColiformTest: out of specifications	Coliform Test: within specifications
Line Sample: gram-negative	Line Sample: gram-positive

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Summary

- The dairy industry needs a better understanding of HRP and their effect on dairy product quality
- Without new technology eliminating HRP is unlikely
- Action can be taken to reduce effects of HRP
- Additional research is required

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Solution for Current Raw Milk Sampling Challenge: The QMI® Aseptic Sampling System

- String Sampling on Dairy Farms
- Bulk Tank or Silo Sampling
- Direct Load Sampling
- Over-The-Road Tanker Truck Sampling
- Identify Sources of Heat Resistant Psychrotrophic Bacteria

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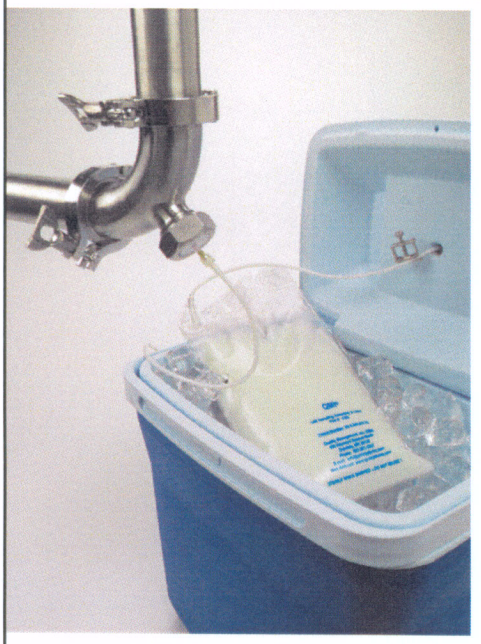

QMI®
Aseptic Sampling System For String Sampling

- Improve Milk Quality
  - somatic cell
  - bacteria counts
- Manage mastitis
  - cultures
- Accurate component analysis
  - fat, protein, etc.

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Needle-only method of sampling through a QMI Aseptic Sampler in an insulated tank door.

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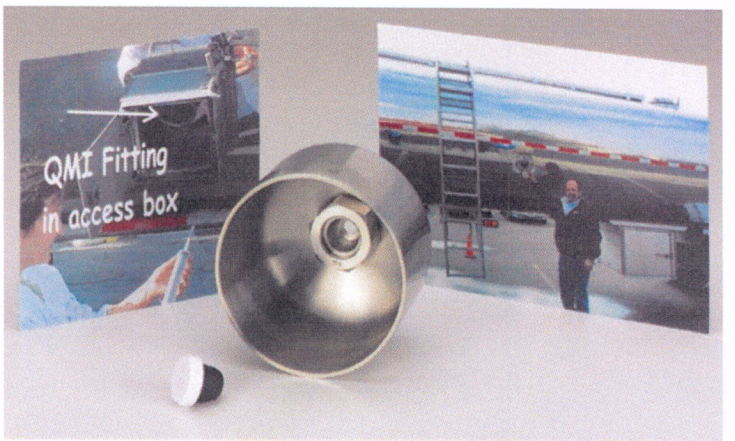

QMI Aseptic Sampling through a port on the side of a tanker truck reduces OSHA risks by not having to climb the ladder to the top hatch.

Shown: PN 7027 (aseptic sampler) and
PN 2238 (wide mouth insulated tank fitting)

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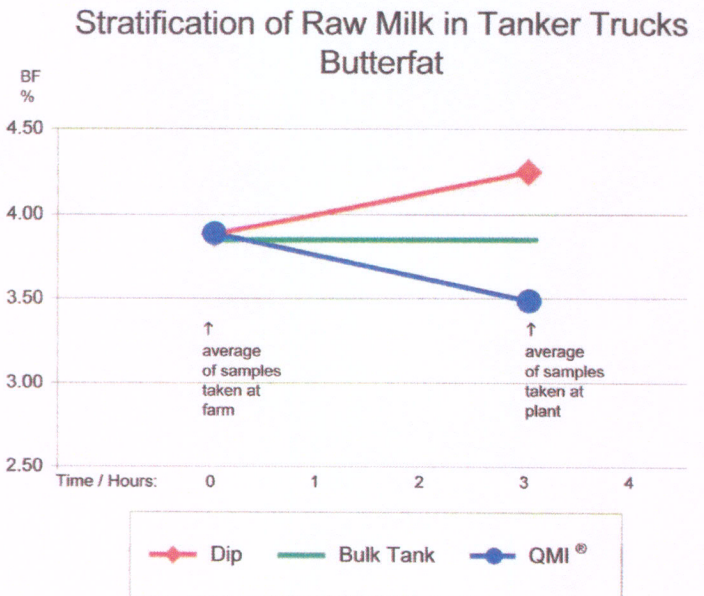

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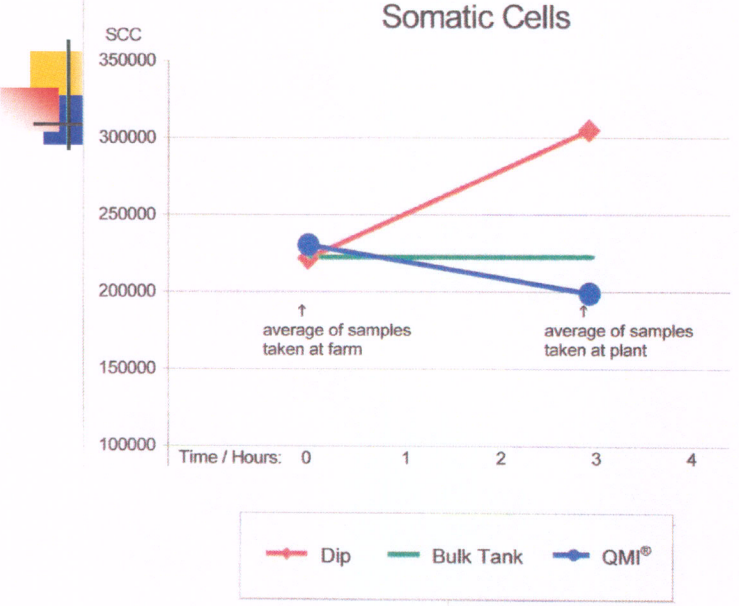
Stratification of Raw Milk in Tanker Trucks
Somatic Cells



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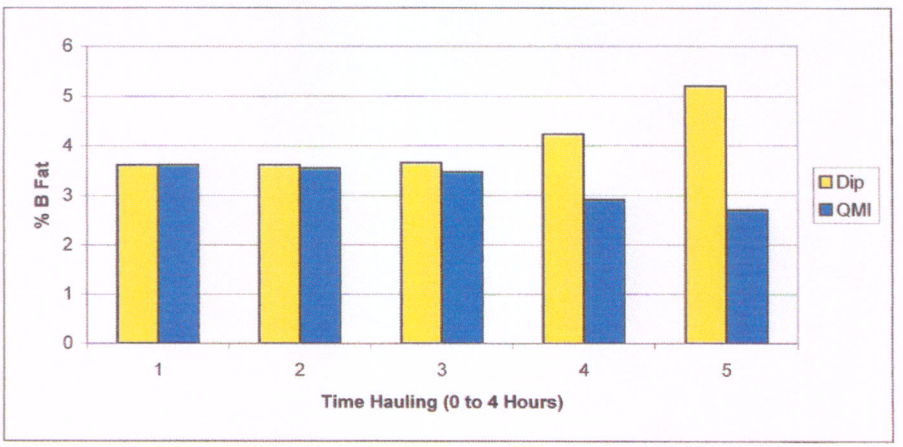
QMI Aseptic Sampling System
Allows Sampling Before Stratification Of Milk During Truck Hauling



To obtain a representative sample, take a QMI Aseptic Sample immediately after filling the tanker truck, before stratification occurs during hauling.

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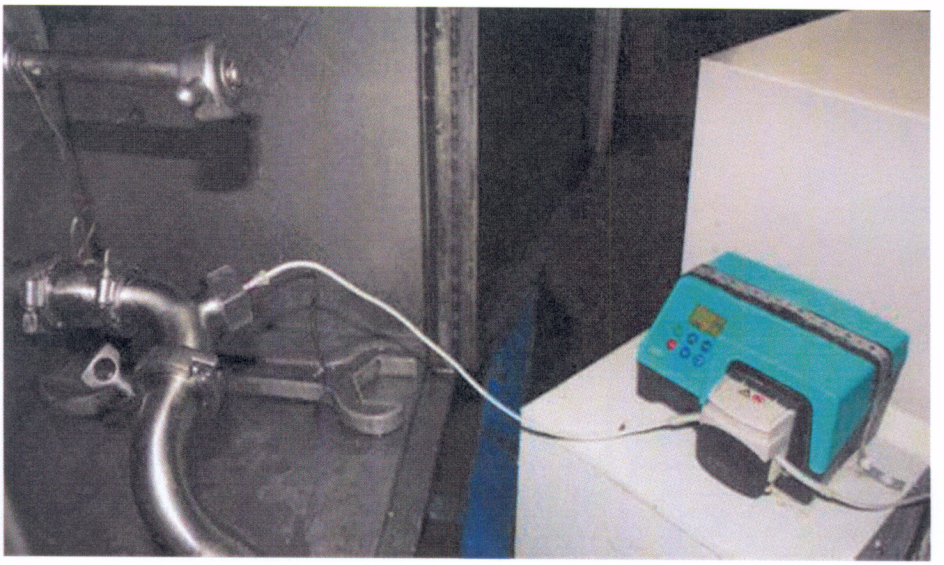

Direct load sampling with peristaltic pump into refrigerator on wheel cart.

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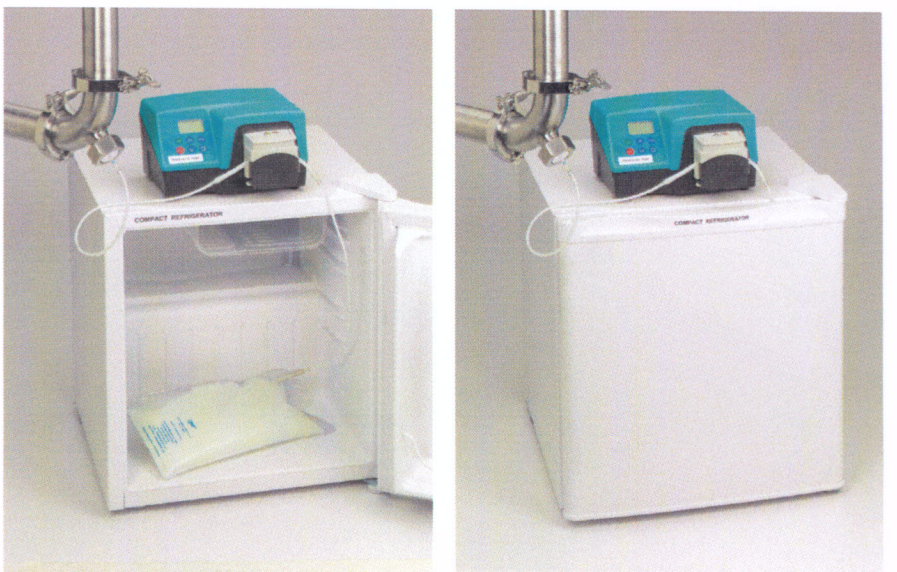

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QMI's Current Research

- QMI® – Quality Management, Incorporated dba QMI conducts on-going research in various aspects of continuous process monitoring.
- For QMI's Newsletter, send your mailing information to QMI
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  by mail: QMI, 426 Hayward Ave. No., Oakdale, MN 55128,
  by fax: 651-501-5797, or by phone: 651-501-2337.
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