MONITORING
MICROBIAL CONTAMINATION OF
DAIRY PRODUCTS
Rev 2007 11 23

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MONITORING MICROBIAL CONTAMINATION
OF DAIRY PRODUCTS

Key Points .
- The dairy industry must be contamination free .
- 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

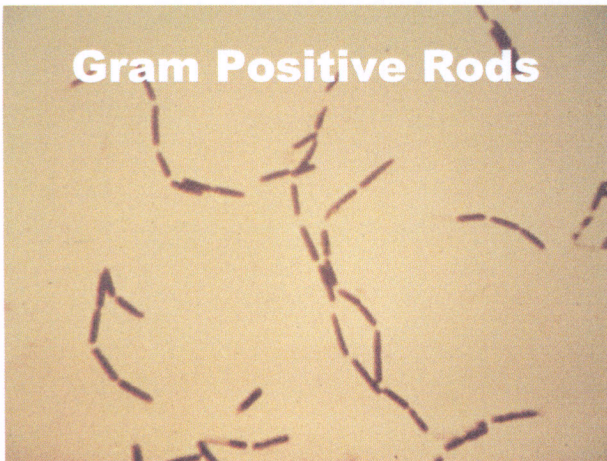| Bacterial Group	Common Source	Common Defect
Gram Negative Rods	Post-Pasteurization Contamination	Bitter, Fruity, Putrid, Unclean, Ropiness
Gram Positive Cocci	Thermoduric and/or Environmental Contamination	Acid Souring, Ropiness
Gram Positive Rods	Thermoduric	Sweet Curdling, Bitter .

. . .

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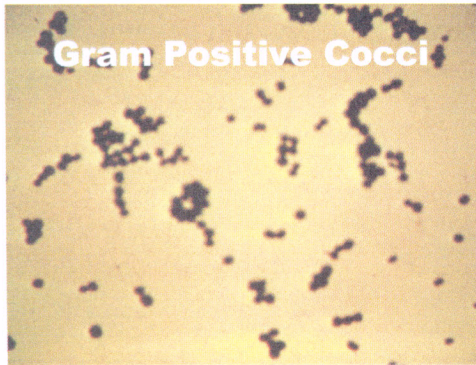## BACTERIAL HAZARDS TO CONTROL IN THE PRODUCTION OF SAFE DAIRY PRODUCTS

- Salmonella
- Yersinia
- Shigella
- Listeria
- Coliforms (pathogenic strains)
- Campylobacter
- Staphococcus
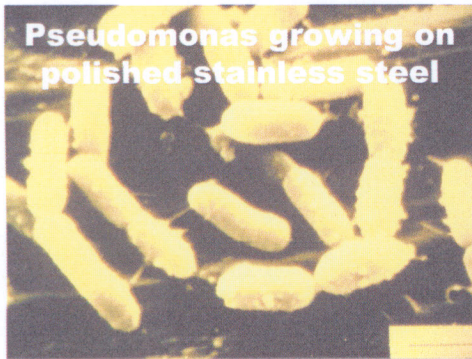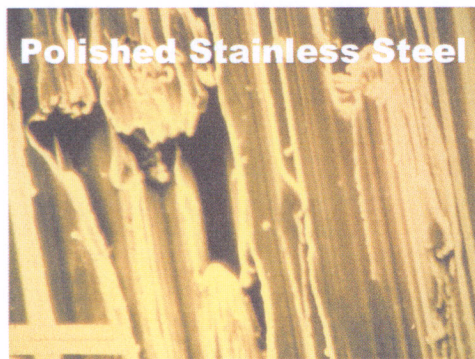- Others

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Gram Positive Rods

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Gram Positive Cocci

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Gram Negative Rods

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Pseudomonas growing on polished stainless steel

9

Polished Stainless Steel

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CONTRIBUTION OF MICROORGANISMS BY
PASTEURIZED STORAGE TANKS
AS DETERMINED BY FILTER COUNTS AND
COLIFORM COUNTS

Range of Counts/100ml	Filter Count/100ml	Coliform Count/100ml
< 1	4	8
1 – 10	4	3
11 – 100	5	1
101 – 1,000	0	0
> 1,000	3	0
Number of samples:	16	12 .

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CONTRIBUTION OF MICROORGANISMS BY
MILK LINES AND *FILLER BOWLS*
## AS DETERMINED BY FILTER COUNTS AND
COLIFORM COUNTS

Range of Counts/100ml	Filter Count/100ml	Coliform Count/100ml
< 1	16	26
1 – 10	10	11
11 – 100	16	1
101 – 1,000	7	3
> 1,000	4	2
Number of samples:	53	43 .

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CONTRIBUTION OF MICROORGANISMS BY
FILLING APPARATUS AND *CARTONS*
AS DETERMINED BY FILTER COUNTS AND
COLIFORM COUNTS

Range of Counts/100ml	Filter Count/100ml	Coliform Count/100ml
< 1	80	99
1 – 10	21	27
11 – 100	28	23
101 – 1,000	35	3
> 1,000	26	1
Number of samples:	190	153 .

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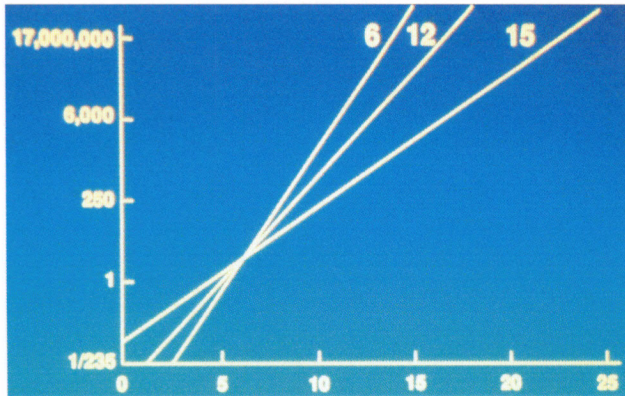
Time to reach a count of 17,000,000 / ml with generation times of 6, 12, and 15 hr and a count of 1 / ml after 5 days of storage.
Marshall, R.T. and R. Appel, 1973. Sanitary conditions in twelve fluid milk processing plants as determined by use of the rinse filler method. J. of Milk and Food Technol.,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

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Factors Affecting
Microbial Contamination (MC)

1. Sanitation

2. Engineering

3. Environmental

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1. Sanitation

- CIP (clean in place) procedures
- Monitor and document clean-up
        (time, temperatures, concentrations)
- Inspect equipment
        (tanks, lines and values)
- Gaskets – line and door
- Valves
- Fittings
- Mandrels
- Defoamers
- Filler sanitation – cleaning, condensation, sanitation .

2. Engineering

- Pasteurized temperature and hold time
- Storage temperatures and hold times for raw milk
- Storage temperatures and hold times for pasteurized milk
- Fill temperature
- Distribution temperature .

2. Engineering, continued

- Line gaskets - cracks
- Fillers (rubber parts)
- Air valves
- Pumps – pinholes
- Cross over piping
- Packaging
- HTST – cracks & pinholes
- Tanks – cracks & pinholes .

3. Environmental

- Water
- Chill water
- Glycol
- Airborne contaminants
- Condensation
- Compressed air: Air blows, Blow molds
- Personnel (hygiene) .

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MICROBIOLOGICAL SURVEY OF GLYCOL SYSTEMS IN MINNESOTA DAIRY PLANTS

Sample #	Coliform MPN / 100ml	Psychrotrophs CFU / ml
1	< 2.2	< 1
2	< 2.2	4,300
3	< 2.2	< 1
4	< 2.2	9
5	< 2.2	< 1
6	< 2.2	< 1
7	< 2.2	420
8	< 2.2	< 1
9	< 2.2	1,100
10	< 2.2	4,800
11	< 2.2	5,400
12	< 2.2	< 1
13	< 2.2	< 1
14	< 2.2	10,000
15	< 2.2	< 1
16	< 2.2	4,800

Data Provided by the Minnesota Department of Agriculture .

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MICROBIOLOGICAL SURVEY OF SWEET WATER SYSTEMS IN MINNESOTA DAIRY PLANTS

Sample #	Coliform MPN/100ml	Psychrotrophs CFU per ml	Sample # cont.	Coliform MPN/100ml	Psychrotrophs CFU per ml
1	2.2	4,300	19	< 2.2	24
2	< 2.2	> 60,000	20	< 2.2	520
3	> 16.0	> 60,000	21	< 2.2	490
4	< 2.2	5	22	9.2	> 60,000
5	< 2.2	< 1	23	> 16.0	> 60,000
6	< 2.2	< 1	24	> 16.0	> 60,000
7	9.2	< 1	25	< 2.2	< 1
8	16.0	18,000	26	> 16.0	> 60,000
9	< 2.2	2,000	27	< 2.2	16
10	< 2.2	63	28	> 16.0	33,000
11	< 2.2	< 1	29	< 2.2	> 60,000
12	> 16.0	< 1	30	< 2.2	< 1
13	< 2.2	> 60,000	31	< 2.2	20
14	< 2.2	< 1	32	< 2.2	> 60,000
15	< 2.2	< 1	33	< 2.2	270
16	< 2.2	650	34	> 16.0	> 60,000
17	< 2.2	3	35	< 2.2	< 1
18	< 2.2	2	36	< 2.2	31,000

Data: Minnesota Department of Agriculture

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COMPARISON OF SPC AND PSYCHROTROPHIC COUNTS ON SWEET WATER SAMPLES FROM TWO MINNESOTA DAIRIES

Dairy	SPC (per ml)	Psychrotrophs (per ml)
# 1	2,700	110,000
# 2	12,000	81,000 .

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HTST

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CONTROL OF THE MICROBIOLOGICAL CONTAMINATION AT HTST FROM DAIRY PLANT COOLANTS IN HTST

- Pressure control when possible
- Anti-microbial treatment programs
- Monitor for pin holes and cracks
- Microbiological testing
  - Psychrotrophs
  - Line sampling – products
    - Aseptic sample
    - 7-day 45° F incubation
    - Gram negative bacteria .

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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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SPC Procedure

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SPOTTY PROBLEMS ?

Contamination Rate   vs.   Percent Spoiled Product

Contamination Rate: 4 bacteria/gallon

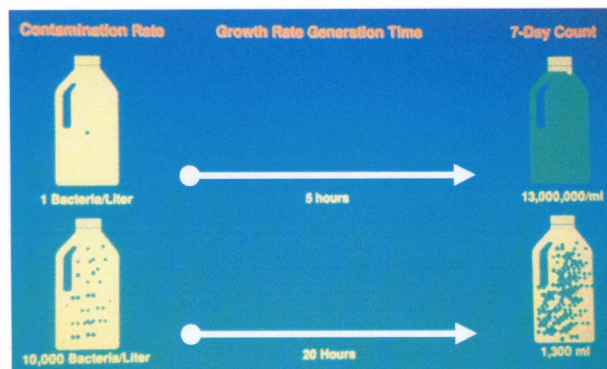- Growth Rate: 5 hours generation time
- Spoilage at 7 days on 25 percent
  of one-half pint product

One gallon =   sixteen ½ pints .

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RELATIONSHIPS AMONG CONTAMINATION RATE, BACTERIAL GROWTH RATE AND PRODUCT SPOILAGE

Contamination Rate   Growth Rate Generation Time   7-Day Count

1 Bacteria/Liter   5 hours   13,000,000/ml

10,000 Bacteria/Liter   20 Hours   1,300 ml

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TRADITIONAL LABORATORY METHODS FOR THE FLUID MILK INDUSTRY

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

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RECOMMENDED LABORATORY METHODS FOR THE FLUID MILK INDUSTRY

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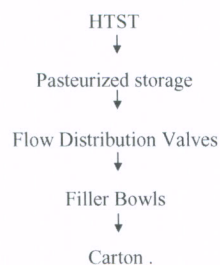 - Chill water
  - Water .

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Appropriate Methods For Sampling and Testing To Monitor MC

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PRODUCTION SEQUENCE ANALYSIS

HTST
↓
Pasteurized storage
↓
Flow Distribution Valves
↓
Filler Bowls
↓
Carton .

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PROPER LINE ANALYSIS

- Large sample size
- Aseptic sample collection
- Proper sample incubation
    Time
    Temperature
    Oxygen
- Proper testing procedures
    Gram negative bacteria
    Gram positive bacteria .

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EFFECT OF OXYGEN AND TEMPERATURE CONDITIONS ON GENERATION TIMES OF *PSEUDOMONAS PUTIDA* IN RAW MILK

Oxygen (ppm)	Temperature (^{o}C)	Generation (h)
1 - 3	3 ± 1	31.0
9 - 12	3 ± 1	16.0
1 - 3	9 ± 1	9.4
9 - 12	9 ± 1	5.4

Data: Brandt & Ledford, Journal of Food Protection (45:2)

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Growth of *Pseudomonas* in milk in QMI® Composite Sampling Bags

Table 1: Daily Cell Counts (cfu / ml)

	Day: 0	3	6	7	8	9	10	13	15	21
Control Bag 1	<1	<1	<1	<1	<1	<1	<1	<1		<1
Control Bag 2	<1	<1	<1	<1	<1	<1	<1	<1		<1
Bag 1	<1	1	15	80	169	334	750	2.2×10^6		6.5×10^7
Bag 2	<1	1	15	70	110	200	350	1.0×10^6		10.5×10^7
Bag 3	<1	2.5	13	70	29	100	600	1.7×10^6		6.0×10^7
Control Syringe 1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Control Syringe 2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Syringe 1	<1	<1	<1	<1	<1	3	4	3	<1	21.5
Syringe 2	<1	<1	<1	<1	<1	2	<1	<1	<1	3.5
Syringe 3	<1	1	<1	1	<1	5	2	1	<1	193

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COMMON MISUNDERSTANDINGS IN INTERPRETATION OF PROCESS (LINE) DATA

- Sample size not related to product volume
- Contamination rate (usually very low)
- Bacterial growth rate
- Psychotropic nature of bacteria
- Statistical significance – any positive test result requires follow-up .

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SHELF-LIFE TEST FOR LINE SAMPLES

- Sampling Procedure:
  - Aseptically obtain product sample, minimum 50 ml, using a QMI ® Aseptic Line Sampler
  - Place sample into a sterile container
  - Provide necessary oxygen for optimum growth rates and accurate test results
- Incubation:
  - Incubate line sample at 7º C / 45º F for 7 - 9 days .

THE DAIRY STRESS TEST

The most useful test for monitoring post-pasteurization contamination
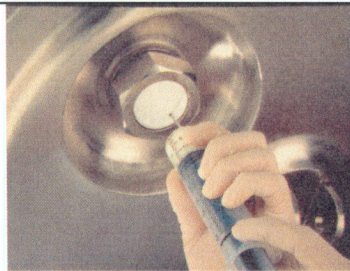
↑ Tank sampling

← Line sampling

The Dairy Stress Test requires aseptic sampling

STRESS TEST ADVANTAGES

Sensitive:
- 1 bacteria / sample
- Optimum pre-incubation temperature for psychrotrophs
- Allows a majority of gram negative organisms to be identified
- Aseptic techniques critical

Selective:
- Gram negative bacteria (indicators of post-pasteurization contamination)

Rapid Results:
- 2 – 3 days

STRESS TEST PROCEDURE

- For tank sampling, aseptically obtain a large product sample, at least 60 ml, using a 60 or 140cc Syringe. Do not incubate in the syringe. Transfer into a QMI ® Composite Sampling Bag or other sterile container.

- For line sampling, aseptically obtain a large product sample using the 2 or 5 liter QMI ® Composite Bag.

- The QMI ® Composite Sampling Bag is oxygen permeable for optimum gram-negative growth and does not require adding head space.

- Incubate sample 24 hours at room temperature *
  * Optional procedure to improve sensitivity and selectivity: add 2.0 ml/50 ml sample of sterile 12.5% sodium deoxycholate (gram-positive inhibitor) .

- Plate using VRB agar - no overlay should be used

- Incubate 72 hours at room temperature, observing for growth each 24 hours .

STRESS TEST RESULTS

- Positive or negative for contamination

- Estimate numbers if possible

- When identifying sources of contamination, any positive test results would indicate contamination .

Effect of Psychrotrophic Sporeforming Bacteria on Quality of Market Milk With Longer Shelf Life
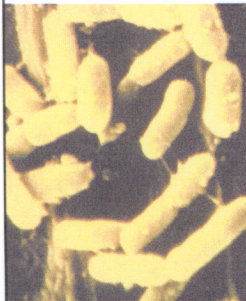
- Raw Milk Quality can influence the keeping quality of market milk
- Psychrotrophic sporeforming bacteria can cause defect in 18 days or more

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.

University of Minnesota Study

Effect of Gram-Positive Psychrotrophic Bacteria on Dairy Product Quality

Sample	Dairy	Bag	Week 3 CFU/ml Gram +	Week 4 CFU/ml 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	6×10
Thursday		B	<10	<10

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

Cornell University Study

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

- 87% of microbial colonies analyzed were gram-positive rods

- 50% of samples: bacteria counts were greater than 1,000,000 CFU/mL after 17 days of refrigerated storage

- *Paenibacillus, Bacillus, Microbacterium*

- *Paenibacillus* appears to be gram-negative in staining procedures, but actually is gram-variable.

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 – may heat shock spores into out growth

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SPORULATION OF GRAM POSITIVE BACTERIA

Raw milk dairy equipment creates sporulation conditions

HTST may heat shock spores into outgrowth
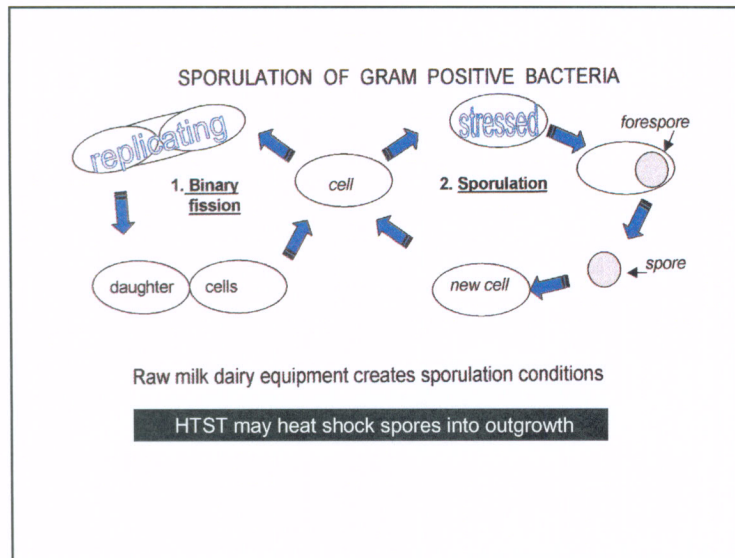
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Raw Milk Handling Equipment: Source of Psychrotrophic Spores

- *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

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Raw Milk Handling Equipment: Source of Psychrotrophic Spores

- 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

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

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 ...

Procedure One, continued:

To determine if psychrotrophic sporeformers 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.

Procedure Two:
QMI^(R) Heat Resistant
Psychrotrophic Bacteria 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

Lab pasteurize QMI^(R) 250ml aseptic sampling bag

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 can occur during washing, sanitizing and drying raw milk equipment - can cause bacteria to sporulate
- Effective sanitation of raw milk handling equipment is often neglected, and
- Contamination rates as low as 1/liter could cause quality defects in pasteurized milk

Microbial Spoilage of Market Milk

Post-Pasteurization Contamination:

Gram-Negative
   Spoilage 10-14 days

Stress Test: positive
Mosely Test: positive
Coliform Test: positive

Line Sample: gram-negative

Heat-Resistant Psychrotrophs:

Gram-Positive
   Spoilage 18+ days

Stress Test: negative
Mosely Test: negative
Coliform Test: negative

Line Sample: gram-positive

Note: more details are available from QMI in the outline titled:

Microbial Spoilage of Market Milk:

Post-Pasteurization Contaminiation, or

Heat-Resistant Psychrotrophic Bacteria

MICROBIOLOGICAL ANALYSIS IS ONLY AS ACCURATE AS THE SAMPLE

The cost of unreliable sampling is the expense
   of unusable data resulting in:
   - Continued microbial contamination
   - Misdirected control efforts
   - Wasted laboratory efforts
   - Regulatory non-compliance
   - Frustrated personnel

The cost of microbiological analysis is too high
   to risk unusable data resulting from
   improperly obtained samples $ $ $.

H A C C P

- QMI® Systems support your effective HACCP Plan:

  - Monitor ingredient quality
  - Monitor critical control points
  - Document process control

QUALITY CONTROL

Basic Principles:

- Quality has to be built into a product and cannot economically or effectively be achieved by inspection alone.

- Quality control oversees the process .

Summary:
MONITORING MICROBIAL CONTAMINATION OF DAIRY PRODUCTS

Key Points .
- The dairy industry must be contamination free .
- 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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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

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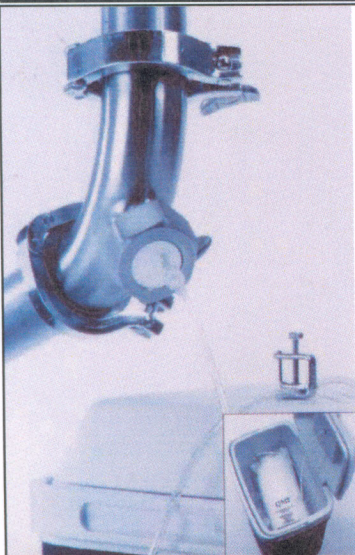
QMI® Aseptic Sampling System For String Sampling

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

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Aseptic Sampling

Needle & Syringe Method

Silo door or tank wall

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QMI®
Composite
Sampling
System

Suggested procedure for
- Direct-load trucks
- String sampling

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QMI®
Successful History

- 30 years of successful sampling
- Customers in thirty countries
- Used in more than 1000 processing plants
- Used on hundreds of dairy farms
- Accepted by farmers and veterinarians for components and herd management

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QMI® System Features

- Aseptic
- University validated
- Allowed by *Standard Methods*
- Conforms to H A C C P principles
- Authorized to use 3A symbol
- Pre-sterilized
- Stable for heat, pressure and length of use
- Easy to install

QMI® Sampling Port Security Covers
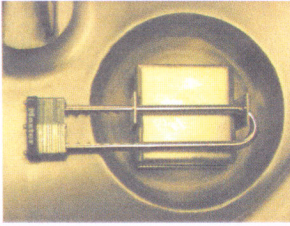
↑ QMI® Locking Cover
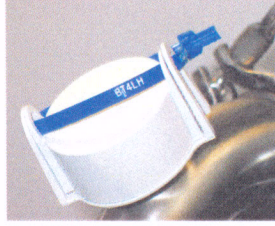
↑ QMI® Tamper Evident Cover
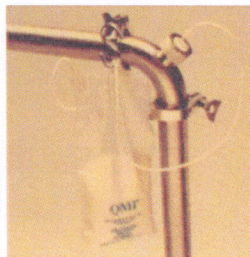With Numbered Tie Strip

QMI® Probe System
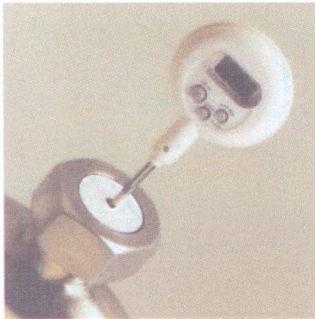
Standard
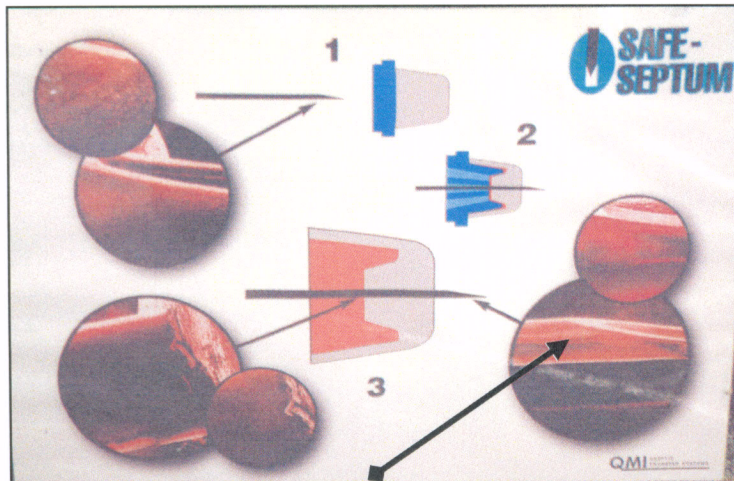Thermometer

or

Recording
Thermometer
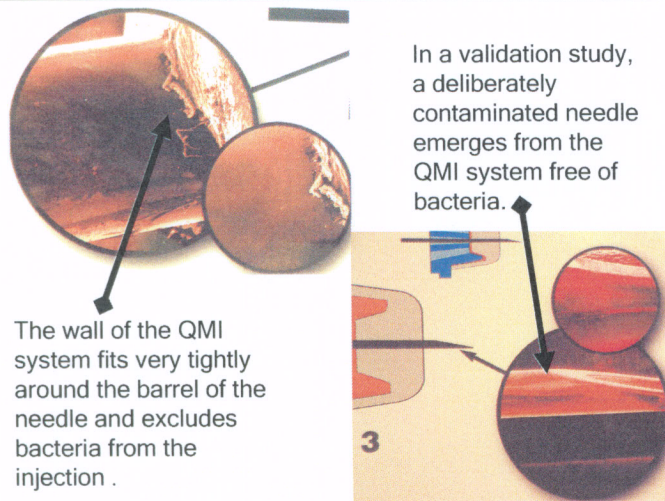
QMI ® Aseptic Sampling System

Used successfully for twenty years

Now in more than one thousand dairy plants worldwide .

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Validation study: deliberately contaminated needle emerges from the QMI system free of bacteria.

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In a validation study, a deliberately contaminated needle emerges from the QMI system free of bacteria.

The wall of the QMI system fits very tightly around the barrel of the needle and excludes bacteria from the injection .

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QMI's Current Research

- QMI – Quality Management, Incorporated conducts on-going research in various aspects of continuous process monitoring.

- For up-dates, ask for QMI's Newsletter. Send your mailing information to QMI by email: info@qmisystems.com, by mail: QMI, 426 Hayward Ave. No., Oakdale, MN   55128, or call: 651-501-2337.

- Visit QMI's website: www . qmisystems . com

This is the end of *QMI's course:*
        Monitoring Microbial Contamination Of Dairy Products.
See also, *QMI's: Raw Milk Sampling Update,*
        Effective Line & Tank Sampling On Dairy Farms, and
        Effects of Heat Resistant Psychrotrophic Bacteria on Market Milk Quality.