Troubleshooting bacteria counts: Organized sampling and testing

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Overall Goals

• We can never keep too many samples when bacteria counts are low or high – duplicate samples

• Understand there is a strategic approach that can be implemented to determine if cows or cleaning or incubation are the issue(s)

• Realize that most times the expertise of multiple parties is needed to permanently resolve the problem

• Use your knowledge along with new technology to help direct you to the area that is causing the issue
Duplicate milk samples

- Keep a duplicate milk sample from every bulk tank load
  - Keep in freezer for one month
- When there is an elevated count then the duplicate sample can be tested
  - GID, LPC, CC
- Why doesn’t taking a bulk tank sample two or three days after an elevated bacteria count work when troubleshooting a bacteria count issue?
- Collect and keep a duplicate bulk tank sample for every load of milk
Routine tasks

**Daily tasks**

- Collect a duplicate bulk tank sample from every load of milk
- Check temperature recorder for...
  - Bulk tank cooling
  - Bulk tank wash
- Check water temperature for wash if recorded
  - Starting temp
  - Divert or end temp

**Weekly tasks**

- Place a mark on the wash chemical barrels to make sure chemical is being used
- Check the marks on the wash chemical barrel to make sure chemical is being used for wash

**Annual tasks**

- Have the wash system analyzed
  - Water temp
  - Chemical titration
  - Slug analysis
  - Water volume
Troubleshooting bacteria counts - Phases

• Phase 1: Elevated or high SPC
• Phase 2: Request differential counts – LPC, PI, GID
• Phase 3: On-farm investigation
• Phase 4: NMC - Troubleshooting Cleaning Problems in Milking Systems
• Phase 5: New technology
Bulk tank milk quality

SCC

Standard Plate Count (SPC)
Living bacteria

Lab Pasteurized Count (LPC)
Bacteria that survive pasteurization - Thermodurics

Preliminary Incubation (PI)
Cold loving bacteria - Psychrotrophs

Coliform Count (CC)
Coliform Bacteria

Mastitis Lab – Bacteria Count (GID)
Identifies each living organism

• All bacteria counts are specific to the conditions that they are incubated under
  – Temperature, time, media, anaerobic, CO2 etc.
• How do the results from a SPC and GID differ?
• How can a SPC and GID be used to support each other?
# Bacteria count comparison

<table>
<thead>
<tr>
<th>Test</th>
<th>Temp °F</th>
<th>Time (hr)</th>
<th>Goal (cfu/ml)</th>
<th>Legal limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC</td>
<td>90</td>
<td>48</td>
<td>≤ 5000</td>
<td>100,000</td>
</tr>
<tr>
<td>LPC*</td>
<td>145</td>
<td>0.5</td>
<td>≤ 250</td>
<td>20,000</td>
</tr>
<tr>
<td>PI*</td>
<td>55</td>
<td>18</td>
<td>&lt; 10,000</td>
<td>no limit</td>
</tr>
<tr>
<td>CC</td>
<td>90</td>
<td>24</td>
<td>≤ 50</td>
<td>no limit</td>
</tr>
<tr>
<td>Mastitis Culture</td>
<td>95 - 100</td>
<td>48</td>
<td>specific to organism</td>
<td>no limit</td>
</tr>
<tr>
<td>Bacteria count - Mastitis lab</td>
<td>95 -100</td>
<td>48</td>
<td>specific to organism</td>
<td>no limit</td>
</tr>
</tbody>
</table>

* Milk sample is subjected to specific environmental conditions (temperature and time) and then incubated as a SPC
Bacteria counts

• How can you use different bacteria count tests together to identify what is responsible for the elevated bacteria count?
SPC + GID + CC + LPC = greater chance to identify bacteria count issue

- Milking wet and/or dirty cows:
  - CC: 100 - 1000 cfu/ml
  - AND LPC < CC
  - AND SPC: 5000 – 20,000 cfu/ml
- Persistent milking machine cleaning problem
  - LPC: 100 - 1000 cfu/ml
  - AND CC < LPC
  - AND SPC: 5000 – 20,000 cfu/ml
- Incubation in the milk handling system
  - CC > 1000 or to numerous to count (TNTC)
  - AND LPC > 100
  - AND SPC > 50,000 or TNTC
- There could be multiple sanitation issues when incubation is identified as the problem
  - Strategic milk sample is required
Milk quality – Troubleshooting
Cleaning Problems in Milking Systems

**SPC**
- Good: 1000 - 5000
- Warning: 10,000
- Action Needed: 100,000

**LPC**
- Good: 10 - 50
- Dirty Equipment: 100
- Total: 500 - 1000

**Coli**
- Good: 10 - 50
- Dirty Cows: 100
- Incubation: 500 - 1000

**SCC**
- Good: 100,000
- Warning: 500,000
- Action Needed: 1,000,000
Visual inspection

• The bacteria counts are only one dimensional
  – You can only assume why the value is what it is if you don’t visit the dairy
• Visit the dairy and look for what should be normal
  – Use of chemicals
  – Water temperature during wash
  – Trapping our during wash
  – Sucking air during wash
  – Slugging action
Wash system analysis

• **NMC - Troubleshooting Cleaning Problems in Milking Systems**
  – Design of milking and wash system
  – Bulk tank milk quality analysis
  – Milk sampling
  – CIP procedures
  – Water quality and quantity
  – Water flow through unit
  – Slug analysis

*Troubleshooting Cleaning Problems in Milking Systems*

From a Paper presented at the 1997 annual meeting of the National Mastitis Council by Douglas J. Reinemann, Ph.D. University of Wisconsin Milking Research and Instruction Lab
# Strategic milk sampling

<table>
<thead>
<tr>
<th>Location/Time</th>
<th>First activation of milk pump</th>
<th>4 hours</th>
<th>8 hours</th>
<th>End of milking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiver 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiver 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer line 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfer line 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk tank 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk tank 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MALDI-TOF MS microorganism identification

Unknown microorganism

Select a colony

Prepare onto a MALDI target plate

Generate MALDI-TOF profile spectrum

Data interpretation

Identified species

Based on proteomics (16s ribosomal proteins), compares mass peaks to known structures for identification
MALDI-ToF

• Swab sections of milking system
  – Near the same areas as strategic sampling
• Send swabs to lab with MALDI-ToF
  – Identify genus and species of organisms on swab
• Use MALDI-ToF on organisms from LPC
  – Identify the heat resistant bacteria
Background on Case Example

- QMPS was contacted by the herd manager to assist in troubleshooting a long standing bacterial count problem
- Milking 1,050 cows
- D-14 parlor
- Milking 3x and washing 2x
- Farm has utilized multiple consultants
Bacteria results

- SPC > 10,000 cfu/ml
- LPC: 1,000
- CC: 40
- What does it mean?
  - LPC 100 - 1000
  - CC < LPC
  - SPC 5,000 – 20,000

Persistent milking machine cleaning issue
Information requested

• Requested
  – Slug analysis
  – Start and end wash water temperature
  – Chemical titration
  – Observation of entire wash cycle
    • Look and listen
  – Sanitize cycle - NO
Further investigation

- MALDI identified
  - *Streptococcus salivarius ssp. thermophilus*
  - Heat resistant bacteria
  - Environmental organism not necessarily associated with a faulty wash system

<table>
<thead>
<tr>
<th>GID</th>
<th>Organism</th>
<th>Count (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/30/2016</td>
<td>Tank 2</td>
<td></td>
</tr>
<tr>
<td><strong>Organism</strong></td>
<td><strong>Count (cfu/ml)</strong></td>
<td></td>
</tr>
<tr>
<td>Strep spp</td>
<td>25,400</td>
<td></td>
</tr>
<tr>
<td>Staph spp</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25,700</strong></td>
<td></td>
</tr>
</tbody>
</table>
Outcome

• It was determined that the chemical pump was not dispensing the proper amount of chemicals during the wash and acid cycles
Discussion

• Use all the tools at your disposal
  – SPC, LPC, CC, Mastitis lab quantitative, NMC Guidelines, MALDI

• Visually inspect the different aspects of the wash system

• Don’t overlook the little things
  – Monitoring chemical usage

• Collect milk samples at different locations from the milking system

• Long-term issues or extreme cases use NMC Troubleshooting Cleaning Problems in Milking Systems Form as a guideline
Questions