USING ON FARM CULTURING TO IMPROVE MASTITIS TREATMENT

How to get started, collect sterile milk samples, culture bacteria and diagnose results
Clinical mastitis is an udder infection that shows symptoms which are visible. The level of infection, or severity, can help herd managers make treatment decisions. The degree of illness and the symptoms present will depend on many factors, such as the nutritional or immune status of the cow, which pathogen is responsible for the inflammation, and a range of environmental factors such as cleanliness, humidity and ambient temperature. Moderate to severe clinical cases can be unpleasant for the cow.

**Non-Severe**

1. **MILD**

   **ABNORMAL MILK**
   Milk has a watery appearance, flakes or clots.

2. **ABNORMAL UDDER**
   Signs of inflammation: swelling, heat, hardness, redness or pain.

**Severe**

1. **2 + 3 = SEVERE**

   **ABNORMAL BEHAVIOR**
   Reduction in milk, fever, lack of appetite, sunken eyes, diarrhea, dehydration or reduction in mobility.
# Clinical Mastitis Treatment Protocol

Large herd management protocol for detection and initial decision making for treatment of clinical mastitis

## Step 1. Detection of Clinical Case
By milking technician in parlor

<table>
<thead>
<tr>
<th><strong>Actions</strong></th>
<th><strong>Milk sample cultured either:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Collect milk sample</td>
<td>1. On-farm</td>
</tr>
<tr>
<td>2. Discard abnormal milk</td>
<td>2. Off-farm</td>
</tr>
<tr>
<td>3. Send cow to hospital after milking</td>
<td></td>
</tr>
</tbody>
</table>

## Step 2. Assign Severity Score
By trained hospital pen manager

<table>
<thead>
<tr>
<th><strong>Severe Case</strong></th>
<th><strong>Non-Severe Case</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms extend beyond udder</td>
<td>Symptoms are restricted to milk and udder</td>
</tr>
</tbody>
</table>

## Step 3. Initial Action
By trained hospital pen manager

<table>
<thead>
<tr>
<th><strong>Immediate symptomatic treatment</strong></th>
<th><strong>Review medical history of cow</strong></th>
</tr>
</thead>
</table>

## Step 4. Initial Decision for Non-Severe Cases
By hospital pen manager at admission to hospital pen before results of culture are known

<table>
<thead>
<tr>
<th><strong>Antibiotic therapy is not likely to be of benefit</strong></th>
<th><strong>Possible benefit of antibiotic therapy</strong></th>
</tr>
</thead>
</table>

## Step 5. Clinical Case Management
By trained hospital pen worker following protocol supervised by attending veterinarian who is responsible for authorizing use of prescription and extralabel drugs.

<table>
<thead>
<tr>
<th><strong>Select a non-antibiotic case management option</strong></th>
<th><strong>Culture-Based Therapy</strong></th>
<th><strong>Option 3:</strong> Symptomatic intramammary RX for 1-3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Option 1: Delayed RX</td>
<td>Option 2: Immediate RX</td>
</tr>
</tbody>
</table>

UW Milk Quality

Dairy Extension

Extension in Education and Extension

Wisconsin
**CLINICAL MASTITIS TREATMENT PROTOCOL**

*Milker detects clinical mastitis. Severity score is assigned.*

**NON-SEVERE CLINICAL CASE**
- Set up on-farm culture.
- Send cow to hospital pen and discard milk.

**SEVERE CLINICAL CASE**
- Do not culture. Treat immediately according to protocol designed by attending veterinarian.

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**DELAYED TREATMENT**
Wait 24 hours for culture results before starting antibiotic therapy.

- **No Growth or Non-Significant Growth**
  - Do not administer intramammary antibiotic unless medical history of cow indicates that her immune system is compromised. Discard milk until it returns to normal (usually 4-6 days).

- **Gram-Negative Growth**
  - Do not administer intramammary antibiotic unless medical history of cow indicates that her immune system is compromised. If intramammary antibiotic is used, ensure that it has a Gram-negative spectrum.

- **Gram Positive Growth Non-Specified Organisms**
  - Give intramammary treatment for 1-3 days using narrow spectrum antibiotic.

- **Gram Positive Growth Likely CNS**
  - Give intramammary treatment for 1-3 days using narrow spectrum antibiotic.

- **Gram Positive Growth Likely *Streptococci spp.***
  - Give intramammary treatment short or long duration depending on medical history of cow.

**IMMEDIATE TREATMENT**
Start short duration intramammary treatment immediately and adjust treatment plan after 24 hours based on culture results.

- **Culture Results at 24 Hours**
  - Stop treatment. Discard milk until it returns to normal and the antibiotic withholding period has ended.

  - **Stop treatment or if medical history indicates antibiotic therapy may be useful, change to an intramammary drug that has Gram-negative spectrum for short duration.**

  - **Stop therapy after completion of 1-3 days of intramammary treatment using narrow spectrum antibiotic.**

  - **Stop therapy after completion of 1-3 days of intramammary treatment using narrow spectrum antibiotic.**

  - **Discontinue intramammary treatment if short duration is appropriate. Continue intramammary treatment if medical history of cow indicates longer duration therapy is justifiable.**

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**Gram Positive Growth Likely *Staph. aureus***
Review medical history of cow before considering treatment. Segregate cow after treatment is completed. Do not treat cows with history of chronic clinical or subclinical infections.
Considerations for use of intramammary (IMM) antibiotics for treatment of non-severe clinical mastitis (severity scores 1 & 2) occurring in cows with a medical history that indicates antibiotic therapy may be justifiable but culture based therapy is not possible.

<table>
<thead>
<tr>
<th>Medical History of Cow</th>
<th>Priority of Treatment Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Watchful Waiting (no IMM antibiotic usage)</td>
</tr>
<tr>
<td>Lactation number</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>Yellow</td>
</tr>
<tr>
<td>≥3</td>
<td>Red</td>
</tr>
<tr>
<td>Number of monthly SCC &gt;200,00 cells/mL</td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>Yellow</td>
</tr>
<tr>
<td>3+</td>
<td>Red</td>
</tr>
<tr>
<td>Stage of lactation (DIM)</td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>Red</td>
</tr>
<tr>
<td>&gt;60</td>
<td>Yellow</td>
</tr>
<tr>
<td>Previous treatment for clinical mastitis in same 1/4</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Yellow</td>
</tr>
<tr>
<td>Yes</td>
<td>Red</td>
</tr>
</tbody>
</table>
On farm culture systems require a designated workspace. Ideally, this should be a clean, well-lit room in a low-traffic area. There should be sufficient counter space and storage that are easily disinfected. Food should not be allowed in this area for health reasons.

**INCUBATOR**

- A small incubator should be purchased to create an environment ideal for bacterial growth.
- Keep the incubator at 37°C or 98.6°F (body temperature).
- A thermometer should be kept inside the incubator at all times, and should be checked daily.
- The humidity should be maintained at 75% by placing a dish of water inside the incubator. Water level should be checked daily and replenished as needed.

**REFRIGERATOR**

- A refrigerator should be purchased for storing media plates and saved milk samples. This refrigerator should not be used for human or animal food.

**EQUIPMENT**

- On-farm cultures use sterile swabs to plate milk samples instead of the sterile loops used in milk quality labs. The estimated plating volume of swabs is 0.1 mL if the swab is dipped in the milk sample for 10 seconds prior to plating.
- Disposable gloves should be worn at all times when handling lab materials.
- Media plates
- Gauze squares soaked in 70% alcohol for disinfecting teats and the counter surface
- Single-use milk sample vials
- Racks for holding sample vials
- Permanent marker
- Biohazard bags
- Bleach
- A cooler with ice for transporting samples from the cow to the lab area.

**PLATE DISPOSAL**

- One more consideration for lab set-up is waste disposal. Use orange biohazard bags whenever discarding infectious materials such as milk samples and culture plates.
- This lab waste must be disposed according to your local regulations. In some locations, plates may be flooded with bleach and then disposed of normally.
- If you have questions about disposal protocol in your area, ask your herd veterinarian.
**Selecting Culture Media**

**Biplate**
Results: Gram-positive, Gram-negative bacteria, No growth or contaminated

- **Factor Agar**
  Growth of Gram-Positive bacteria

- **MacConkey Agar**
  Growth of Gram-Negative bacteria

**Triplate**
Results: Staphylococcus spp., Streptococcus spp., Gram-negative bacteria, No growth or contaminated

- **Factor Agar**
  Growth of Gram-Positive bacteria

- **MacConkey Agar**
  Growth of Gram-Negative bacteria

- **Modified TKT (MTKT) Agar**
  Growth of Streptococcus

**Quad plate**
Results: Staphylococcus spp., Streptococcus spp., Gram-negative bacteria, No growth, contaminated or others.

- **Blood Agar**
  Growth of most bacteria

- **Factor Agar**
  Growth of Gram-Positive bacteria

- **MacConkey Agar**
  Growth of Gram-Negative bacteria

- **Modified TKT (MTKT) Agar**
  Growth of Streptococcus
The entire process of performing on farm culturing is absolutely dependent on how well the milk sample was collected. If this step is not performed correctly, then the rest of the process will be meaningless. Follow these steps to help collect a sterile milk sample.

1. After detecting the mastitis case, label a sterile (unopened) vial with the cow ID, quarter and date. Immediately cool sample in a cooler or refrigerator until plating can occur.

2. Dry wipe udder to remove loose debris.

3. Apply predip with 20-30 second contact time to sanitize teat skin.

4. Dry off teat with single-use towel.

5. Forestrip 3-4 streams of milk to remove bacteria from teat canal.

6. MOST CRITICAL: Scrub end of teat with gauze swab soaked in 70% alcohol.

7. Collect milk sample into the labeled vial.
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2. Dry off teat with single-use towel.

3. Apply predip with 20-30 second contact time to sanitize teat skin.

4. Dry off teat with single-use towel.

5. Forestrip 3-4 streams of milk to remove bacteria from teat canal.
HOW TO COLLECT A STERILE MILK SAMPLE

6 MOST CRITICAL
Scrub end of teat with gauze swab soaked in 70% alcohol.

7 Collect milk sample into the labeled vial. Do not touch any inner part of the vial to avoid contamination of the sample.

8 Immediately cool sample in a cooler or refrigerator until plating can occur.