1. PURPOSE

To detect and treat mammary infections quickly and appropriately in cows with;

- High somatic cell count
- Clinical and sub clinical mammary infections
- Fresh cows (as early as 7th milking postpartum)

2. RESPONSIBILITY

2.1 Trained full time or casual staff.

3. GENERAL

3.1 Collect aseptic milk samples as per SOP DC-615: Milk Sampling.

3.2 CMT = California mastitis test

4. MATERIAL

4.1 Disinfectant spray for surfaces
4.2 Paper towel
4.3 Dish soap
4.4 Clean dish/container
4.5 Sterile sample tube
4.6 Test tube holder
4.7 Sharpie
4.8 Butterfields buffer dilution tubes (9ml)
4.9 Sterile pipettes
4.10 Pipette bulb
4.11 Incubator (White foam egg incubator set at 35 degrees C and or CheckUp incubator set at 37 degrees C)
4.12 Spreader
4.13 3M Petrifilm plates: Aerobic (AC), Coliform (CC), Staph Express Count Plate (STX), Staph Express Disk (stored in pharmacy freezer)
4.14 Hand sanitizer
4.15 1 ml sterile syringe
4.16 Checkup Petri dish (kit)

5. PROCEDURES

5.1 PREPARATION:

5.1.1 Plug in the incubator(s) you will be using to warm them up.
5.1.2 Disinfect the work surface with spray.
5.1.3 In a clean dish;

5.1.3.1 Add a drop of dish soap to cold/lukewarm water.
5.1.3.2 Insert the milk sample tubes to wash the exterior. This is to minimize contamination of the cultures.

5.1.3.3 Rinse the tubes

5.1.3.4 Place the tubes in the test tube holder. Separate any quarters that have clinical mastitis or have tested positive on the CMT.

5.1.4 Prepare all other materials needed (buffer, empty sample tube, spreader, sharpie, pipettes, bulb).

5.1.5 Calculate the number of Petrifilm Plates required for the cultures.

A. **Pooled culture:** Pool the milk from all quarters testing negative on the CMT or without mastitis.
   Plates required: 1 x STX plate.

B. **Individual culture:** Milk from any quarter testing positive on the CMT or with mastitis.
   Plates required: 1 x STX, 1x CC, 1 x AC per infected quarter.

5.2 Disinfect hands with hand sanitizer before removing any Petrifilm plates from their respective packages.

5.3 Place them on the clean work surface.

5.4 Label each Petrifilm with:
   - Cow ID # (top left)
   - Dilution (top middle)
   - Quarter # or "pool" (top right)
   - Date (bottom left)
   - Time (bottom right)

5.5 **POOLED SAMPLE: (Dilution 1:10)**

5.5.1 Shake the milk in each tube.

5.5.2 Loosen the caps.

5.5.3 Place pipette bulb on the end of the sterile pipette.

5.5.4 Using the same pipette for each sample, remove 1 ml of milk and add to empty sterile sample tube. Shake well.

5.5.5 Change the pipette.

5.5.6 Open a Butterfields Buffer Dilution tube. Add 1 ml of the mixed or "pooled" milk to the buffer.

5.5.7 Aspirate this solution several times into and out of the tube to flush the pipette. Shake well.

5.5.8 Inoculate 1 ml of the diluted milk in the middle of the STX PETRIFILM without touching it with the pipette.

5.5.9 Slowly roll down the top film to remove any air bubbles.

5.5.10 Place the flat side of the spreader in the center of the plate and push down to spread the diluted milk evenly on the plate.

5.5.11 If the pooled sample comes back positive ( > 10 colonies) within 24 hours, thaw the milk samples in lukewarm water and plate individually as per instructions below.

5.6 **INDIVIDUAL SAMPLE:** The dilution depends on the severity of the infection represented by the CMT gel viscosity upon reaction;
<table>
<thead>
<tr>
<th>VISCOSITY</th>
<th>RESULT</th>
<th>DILUTION</th>
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<tbody>
<tr>
<td>Liquefied</td>
<td>Negative</td>
<td>N/A</td>
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<tr>
<td>Light gel</td>
<td>Positive</td>
<td>1:10</td>
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<tr>
<td>Medium gel</td>
<td>Positive</td>
<td>1:100</td>
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<tr>
<td>Thick gel</td>
<td>Positive (Clinical Mastitis)</td>
<td>1:1000</td>
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5.6.1 Disinfect your hands and work surface between each Milk sample (not the Petrifilm but the actual milk sample).

5.6.2 For 1:100 dilution:
5.6.2.1 Using a 1ml syringe, remove 0.1 ml of milk and add to the buffer. Shake well.
5.6.2.2 Aspirate this solution several times into and out of the tube to flush the pipette. Shake well.

5.6.3 For 1:1000 dilution:
5.6.3.1 Using a 1 ml syringe remove 0.1 ml of milk and add to the Butterfield’s buffer. Shake well.
5.6.3.2 Using a sterile pipette transfer 1ml of that solution to a second Butterfield’s buffer.
5.6.3.3 Aspirate this solution several times into and out of the tube to flush the pipette. Shake well.

5.6.4 Place a sterile pipette on the bulb in preparation for plate inoculation;

5.6.5 STX Petrifilm:
5.6.5.1 Inoculate 1 ml of the diluted milk in the middle of the STX Petrifilm without touching it with the pipette.
5.6.5.2 Slowly roll down the top film to remove any air bubbles.
5.6.5.3 Place the flat side of the spreader in the center of the STX plate and push down to spread diluted milk evenly on the plate.

5.6.6 CC Petrifilm:
5.6.6.1 Using the same pipette, inoculate 1 ml of the diluted milk in the middle of the CC Petrifilm without touching it with the pipette.
5.6.6.2 Slowly roll down the top film to remove any air bubbles.
5.6.6.3 The CC plate spreads itself evenly on its own.

5.6.7 AC Petrifilm:
5.6.7.1 Using the same pipette, inoculate the AC plate and drop the top film down over the sample.
5.6.7.2 Turn the spread over to the side with the ridge.
5.6.7.3 Place over the center of the inoculated milk and press down to form a perfect circle.

5.7 Place all Petrifilms in the incubator. They can be stacked one on top of the other (max 10)

5.8 Rinse/wash all equipment and return.

5.9 Dispose of used pipettes and diluted milk. Return bag of 3M Petrifilms to the freezer. Milk samples are frozen in case we need to retest at a later date.

5.10 Check the incubator temperature throughout the day. Adjust the temperature by opening or closing the air vent at the top if necessary to maintain at 35°C.

5.11 Check the Petrifilms in 24 hours.

5.12 INTERPRETATION OF RESULTS:
5.12.1 STX Petrifilm;
5.12.2 CC Petrifilm:

- **Blue Colonies**
  - not Staphylococcus but other Gram (+) bacteria.

- **Black or Purple Colonies**
  - Pink halos > 1 mm in diameter around the colonies
  - Pink halos < 1 mm in diameter around the colonies

**STEP 1:**
Disinfect hands

**STEP 2:**
Gently peel back the top film of the STX Petri film and insert the blue Staph express disk.

**STEP 3:**
Drop the top film of the STX Petri film back down and smooth over.

**STEP 4:**
Wait up to 3 hours for results

**Results in 24 hours or more.**

Reference the Culture Interpretation Guide section of the instruction manual to interpret the results.

.Dispose of bag with Petri dish in the bio box.

**Record the observations in the Treatment log book (cow #, quarter # or pooled sample, dilution, result, # of colonies present).**

5.13 Record the observations in the Treatment log book (cow #, quarter # or pooled sample, dilution, result, # of colonies present)

5.14 **CHECKUP PETRI DISH CULTURES:** For use when difficult to interpret the results of the 3M Petrifilms.

- **STEP 1:**
  - Disinfect hands

- **STEP 2:**
  - Gently peel back the top film of the STX Petri film and insert the blue Staph express disk.

- **STEP 4:**
  - Wait up to 3 hours for results

- **STEP 4:**
  - Pink halos > 1 mm in diameter around the colonies
  - Pink halos < 1 mm in diameter around the colonies

**If interpretation is difficult, or identification of bacterial strain is required, perform a Checkup Petridish Culture. See Section 5.14**

5.14.1 Plug in the Check Up incubator

5.14.2 Use aseptic techniques.

5.14.3 Follow the plating protocol as per described on pages 11 and 12 of the Checkup Instruction manual.

5.14.4 Place the Petri dish in a Ziploc bag. Seal and place it in the “Checkup” incubator at 37 degrees Celsius. Results in 24 hours or more.

5.14.5 Reference the Culture Interpretation Guide section of the instruction manual to interpret the results.

5.14.6 Dispose of bag with Petri dish in the bio box.

5.14.7 Record the observations in the Treatment logbook (cow #, quarter # or pooled sample, dilution, result, # of colonies present).

5.15 Discuss treatment options and with head technician or dairy manager. Treatment depends on the pathogen and the # colonies present.
## Document Status and Revision History

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<th>DATE</th>
<th>PREVIOUS VERSION</th>
<th>NEW VERSION</th>
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