MILK CULTURING

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 and qualified personnel.

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 or pipette tips
4.10 Micro pipette or 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 Petridish
4.15 Hand sanitizer
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, pipette and tips.

5.1.5 Calculate the number of Petrifilm Plates and/or Petridish 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 (Subclinical mastitis): Milk from any quarter testing positive on the CMT. Plates required: 1 x STX, 1x CC, 1 x AC per infected quarter.

C. Individual culture (Clinical mastitis): Milk from infected quarter with mastitis. Plates required: Petridish.

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; (Fig.1)

- Cow ID # (top left)
- Dilution (top middle)
- Quarter # or "pool" (top right)
- Date (bottom left)
- Time (bottom right)

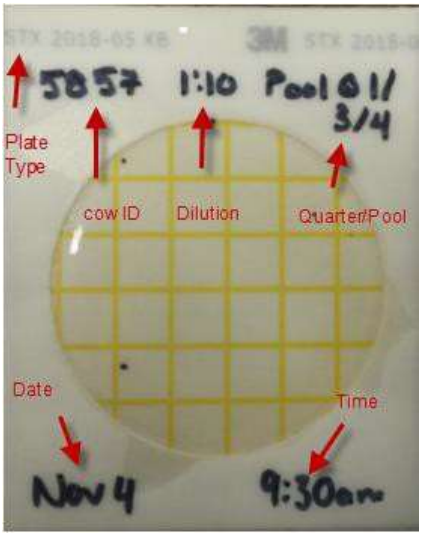


Figure 1: Label Petrifilm

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 sterile pipette tip on micro-pipette calibrated at 1000µl.

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

5.5.5 Change the pipette tips.

5.5.6 Open a Butterfields Buffer Dilution tube. Add 1000µl (or 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 tips. 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 (> 15 colonies) within 24 hours, thaw the milk samples in lukewarm water and plate individually as per instructions below.

5.6 INDIVIDUAL SAMPLE:

5.6.1 Individual samples can be processed using Petridish or Petrifilm

5.6.1.1 Petridish: No dilution is required.

5.6.1.2 Petrifilm: The dilution depends on the severity of the infection represented by the CMT gel viscosity upon reaction. Refer to Section 5.6.2 and **Table 1**: Petrifilm dilution according to Viscosity

TABLE 1: Petrifilm Dilution According to Viscosity

VISCOSITY	RESULT	DILUTION
Liquefied	Negative	1:10
Light gel	Positive	1:10
Medium gel	Positive	1:100
Thick gel	Positive (Clinical Mastitis)	1:1000

5.6.2 Disinfect your hands and work surface between each Milk sample (not the Petrifilm but the actual milk sample).

5.6.1.3 For 1:10 dilution

5.6.1.2.1 Using a pipette, remove 1.0 ml of milk and add to the Butterfield's buffer.

5.6.1.2.1 Shake well.

5.6.1.4 For 1:100 dilution:

5.6.1.2.1 Using a pipette, remove 1.0 ml of milk and add to the buffer.

5.6.1.2.1 Shake well.

5.6.1.2.1 Transfer 1.0ml of this solution and add to a second Butterfield's buffer.

5.6.1.2.1 Shake well.

5.6.1.5 For 1:1000 dilution:

5.6.1.2.1 Using a pipette, remove 1.0 ml of milk and add to the Butterfield's buffer.

5.6.1.2.1 Shake well.

5.6.1.2.1 Transfer 1ml of that solution to a second Butterfield's buffer.

5.6.1.2.1 Shake well.

5.6.1.2.1 Transfer 1ml of that solution to a third Butterfield's buffer.

5.6.1.2.1 Shake well.

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

5.6.4 Place a sterile pipette tip on the micro-pipette or pipette bulb in preparation for plate inoculation.

5.6.1.6 STX Petrifilm:

5.6.1.2.1 Inoculate 1 ml of the diluted milk in the middle of the STX Petrifilm without touching it with the pipette tip.

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

5.6.1.2.1 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.

5.6.1.7 CC Petrifilm:

5.6.1.2.1 Using the same pipette tip, inoculate 1 ml of the diluted milk in the middle of the CC Petrifilm without touching it with the pipette.

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

5.6.1.2.1 The CC plate spreads itself evenly on its own.

5.6.1.8 AC Petrifilm:

5.6.1.2.1 Using the same pipette tip, inoculate the AC plate and drop the top film down over the sample.

5.6.1.2.1 Turn the spread over to the side with the ridge.

5.6.1.2.1 Place over the center of the inoculated milk and press down to form a perfect circle.

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

5.6.6 Rinse/wash all equipment and return.

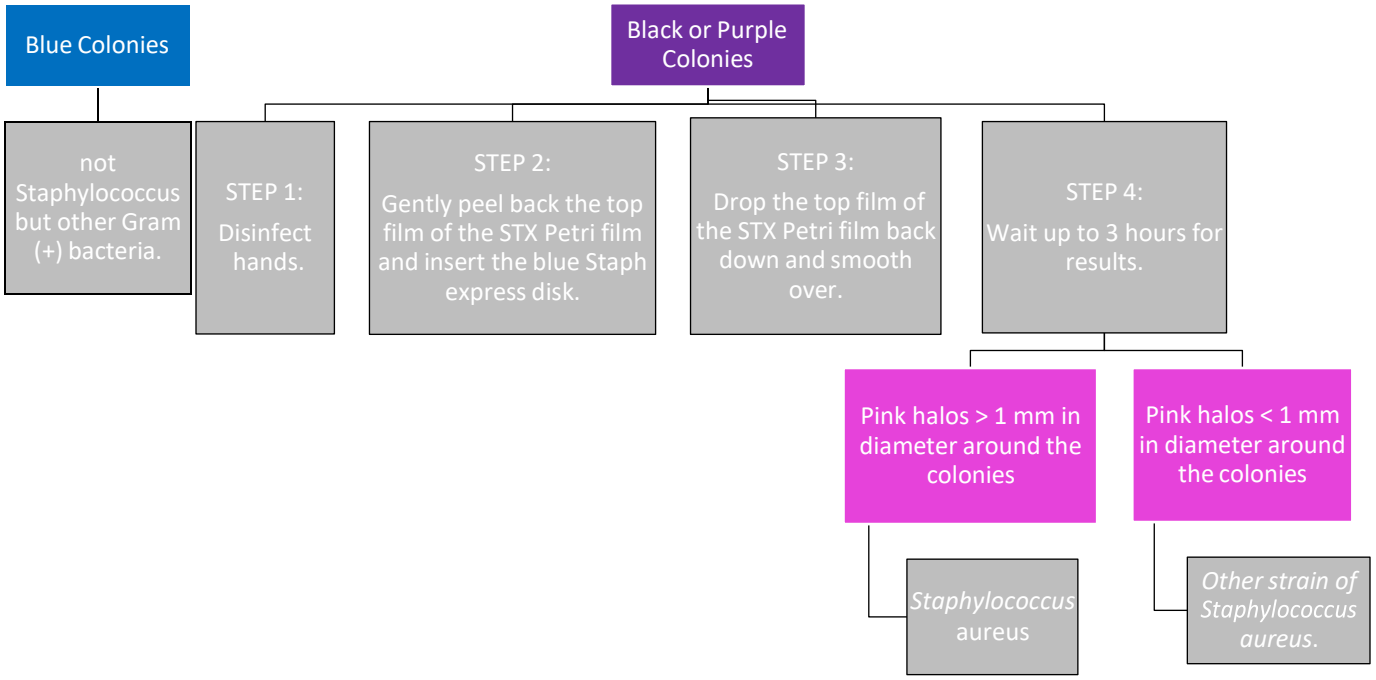
5.6.7 Dispose of used pipette tips and diluted milk. Return bag of 3M Petrifilm to the freezer. Milk samples are frozen in case we need to retest at a later date.

5.6.8 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.

5.6.9 Check the Petrifilm in 24 hours.

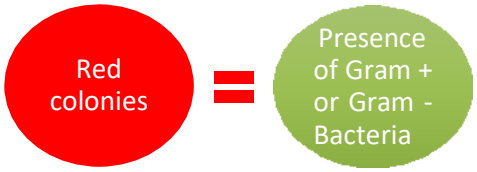
5.7 <u>INTERPRETATION OF RESULTS:</u>

5.7.1 STX Petrifilm.



5.7.2 CC Petrifilm:

5.7.3 AC Petrifilm



If interpretation is difficult, or identification of bacterial strain is required, perform a Checkup Petridish Culture. See Section 5.7

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

5.7 CHECKUP PETRI DISH CULTURES: For use when difficult to interpret the results of the 3M Petrifilm and for clinical mastitis.

5.7.1 Plug in the Check Up incubator.

5.7.2 Use aseptic techniques.

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

5.7.4 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.

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

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

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

5.8 Discuss treatment options and with Technician or Herd Manager. Treatment depends on the pathogen and the # colonies present.

Document Status and Revision History

DATE	STATUS
8-Feb-2018	Version 01: MAC Campus FACC approved
22-aug-2023	Version 02: MAC Campus FACC approved