Colostrum Culture Plating Procedure

(If multiple samples—raw, pasteurized, as fed, etc.—label the plates appropriately)

1. Thaw sample at room temperature or in a cool water bath.

2. Set out one of each of the following plates: Factor, TKT, and MacConkey. Label as needed. Allow plates to warm to room temperature for at least 5 minutes.

3. Place 19.8 ml of sterile water into a 50 ml sterile cup. (Only use sterile syringes and needles to measure the sterile water.)

4. Vortex the colostrum sample, then pipette 0.2 ml of colostrum into the cup of sterile water.

5. Vortex the cup, then pipette 0.2 ml of the diluted colostrum onto each plate.

6. Save the colostrum sample in the freezer in case it needs to be re-plated at a different dilution.

7. Evenly streak the colostrum over each of the 3 plates using a sterile loop. Streak over the entire surface of the agar in a left to right direction, and then repeat in an up and down direction to be sure that the whole plate is covered.

8. Incubate at 37°C with plates inverted.

Colostrum Culture Reading Procedure

1. Identify and count the colonies present at 24 hours as follows. (Over 250 colonies is TNTC- too numerous to count. The colostrum can be re-plated at a 1:5,000 dilution in order to get accurate colony counts, if desired by the client or veterinarian. Likewise, if there are very few or no colonies present, the colostrum can be re-plated at 1:5 and 1:50 dilutions.)

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- **Factor Plate:**
    - Staph aureus- beta-hemolytic, yellow or white, catalase-positive; confirm with positive coagulase test (If coagulase test is negative, add any colonies that were counted as Staph aureus to the count of coagulase-negative Staphs, and change the count of Staph aureus to zero.)
    - Coagulase-negative Staph- non-hemolytic, yellow or white, catalase-positive
    - Strep species- gray colonies, catalase-negative (ignore on the Factor plate; these will be counted on the TKT plate)
    - Others (yeast, Prototheca, Bacillus, etc.)- identify and confirm as you would for an individual cow milk culture
- **TKT Plate:**
    - Strep agalactiae- beta-hemolytic, CAMP-positive; re-streak colony onto a new TKT plate, incubate overnight, and ship to the University of Minnesota Udder Health Lab for confirmation via API or bacterial sequencing (If CAMP test is negative, add any colonies that were counted as Strep ag. to the count of Strep species, and change the count of Strep ag. to zero.)
    - Strep species- non-hemolytic
- **MacConkey Plate:**
    - Coliforms- pink, opaque
    - Non-coliform gram-negatives- translucent

2. Repeat the colony counts after 42-48 hours of incubation.

3. Multiply the 42-48 hour colony counts by 500 to determine CFU/ml for each organism. (If sample was re-plated at 1:5, 1:50, or 1:5,000 dilutions, multiply by 5, 50, or 5,000, respectively.)

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