Bulk Tank Milk Culture Plating Procedure

(Aerobic culture only; does not include a Mycoplasma culture)

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

2. Set out one of the following per farm/tank: 100ml sterile urine cup, Factor plate, TKT plate, and MacConkey plate. Label the cups and plates with farm number or initials (and tank number if applicable). Allow plates to warm to room temperature for at least 5 minutes.

3. Vortex all of the samples from a particular farm/tank, then mix them together in the sterile cup to create a composite sample for that farm/tank.

4. Vortex the composite sample, then pipette 0.2 ml onto each plate (Factor, TKT, and MacConkey).

5. Evenly streak the milk over each of the 3 plates using a sterile loop. Streak the milk 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.

6. Place the composite sample in the freezer and do not discard for at least 1 week.

7. Incubate at 37°C with plates inverted.

Bulk Tank Milk 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 sample can be re-plated at 1:50 and 1:500 dilutions in order to get accurate colony counts, if desired by the client or veterinarian.)

    - **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.)

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- 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 5 to determine CFU/ml for each organism. (If sample was re-plated at 1:50 or 1:500 dilutions, multiply by 50 or 500, respectively.)

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