Towel Culture Plating Procedure

1. If towel is frozen, sit out to thaw (without removing it from its bag).

2. Sterilize an area of the counter with alcohol. Let dry.

3. With gloved hands, remove the towel from the bag and lay flat on the sterilized area of counter. Measure the towel (in inches) with a ruler. Multiply the towel's length by its width to determine the area of the towel in square inches.

4. With gloved hands, fold the towel and place into a Whirl-Pak bag.

5. *Note*: open a new bottle of sterile water and use a new syringe and new needle. Add to the Whirl-Pak bag an amount of sterile water in ml equal to the area of the towel in square inches. (example: if towel area = 60 sq. in., add 60ml of water)

6. Close the Whirl-Pak bag and knead so that the towel absorbs the water. Let soak for 10+ minutes.

7. In the meantime:

    - Get 2 Factor & 2 MacConkey plates out of the fridge. Label one of each as 5, and the other as 50.

    - Get out 2 plastic 3ml tubes; label one as 5 and the other as 50. Add 2ml of sterile water to tube 50.

8. Knead the Whirl-Pak bag again, then squeeze out and pour approximately 1ml of water from the bag into tube 5.

9. Vortex tube 5, then pipette 200ul from tube 5 into the tube labeled as 50.

10. Vortex tube 50, then pipette 200ul from tube 50 onto each plate labeled as 50. Spread evenly over the agar with a sterile loop.

11. Vortex tube 5, then pipette 200ul from tube 5 onto each plate labeled as 5. Spread evenly over the agar with a sterile loop.

12. Incubate at 37°C for 18-24 hours with plates inverted.

Towel Culture Reading Procedure

Factor plate:

1. Decide which Factor plate (5 or 50) has 25-250 total colonies growing. This dilution will be used for gram-positive colony counts. (If there are fewer than 25 colonies, use the dilution with the most colonies. If there are more than 250 colonies on both dilutions, the sample will have to be further diluted and re-plated. Ask Joanna for instructions on how to do this.)

2. Identify and count the colonies as follows. If you are unsure, please ask Joanna to help. Streps and Staphs must be differentiated by catalase-testing each colony. (Use a clean toothpick to transfer some of a colony into a drop of hydrogen peroxide. The colony is catalase-positive if bubbling occurs.)

    - ***Bacillus:*** large, flat, rough colonies
    - ***Yeast:*** white, waxy colonies (perform a gram-stain to confirm)
    - ***Prototheca:*** tiny, gray, dry colonies (perform a gram-stain to confirm)
    - ***Streps:*** shiny gray or white colonies (catalase-negative)
    - ***Staphs:*** shiny gray, white, or yellow colonies (catalase-positive)

3. Multiply the count of each type of colony by the dilution factor of the Factor plate, to determine the number of each organism per square inch of towel.

MacConkey plate:

1. Decide which MacConkey plate (5 or 50) has 25-250 total colonies growing. This dilution will be used for gram-negative colony counts. (If there are fewer than 25 colonies, use the dilution with the most colonies. If there are more than 250 colonies on both dilutions, the sample will have to be further diluted and re-plated. Ask Joanna for instructions on how to do this.)

2. Identify and count the colonies as follows.

    - ***Coliforms:*** pink, opaque colonies
    - ***Non-coliform gram-negatives:*** translucent colonies

3. Multiply the count of each type of colony by the dilution factor of the MacConkey plate, to determine the number of each organism per square inch of towel.