# Bedding Culture Plating Procedure

(If multiple samples are submitted, i.e. clean vs. stall sand or pen 1 vs. pen 2 sand, label the tubes and plates appropriately)

1. If sample is frozen, sit out on the counter to thaw.

2. Pour bedding into a 50ml sterile urine cup and pack lightly with gloved fingers until it is level at the 50ml mark.

3. Place this measured sample into a Whirl-Pak bag, then dispose of the 50ml cup.

4. Open a new 250ml bottle of sterile water, pour it into the Whirl-Pak bag, close the bag, and then mix the bedding and water by shaking the bag.

5. Allow to soak for 10+ minutes.

6. In the meantime:

    - Get 4 Factor & 4 MacConkey plates out of the fridge. Label as plates 0—3.
    - Label 4 plastic 3ml tubes as 0—3.
    - Open a new 20ml bottle of sterile water, and using a new 3cc syringe and new needle, add 2ml of sterile water each to tubes 1—3. (Do not add any to tube 0.)

7. Shake the Whirl-Pak again, then use a pipette to transfer 600ul of liquid into tube 0. (Avoid sucking up bedding particles with the liquid).

8. Vortex tube 0, then pipette 200ul from tube 0 to tube 1.

9. Vortex tube 1, then pipette 200ul from tube 1 to tube 2.

10. Vortex tube 2, then pipette 200ul from tube 2 to tube 3.

11. Starting with tube 0, vortex, then pipette 200ul onto each plate 0, then spread evenly over the agar with a sterile loop. Repeat with tubes 1—3 and plates 1—3, respectively.

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

# Bedding Culture Reading Procedure

**Factor plate:**

1. Decide which Factor plate (0, 1, 2, or 3) has 25-250 total colonies. 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 all dilutions, the sample will have to be further diluted and re-plated. Ask Joanna for instructions.)

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. The dilution factors of each plate are:

   - 0: 1:5 dilution (multiply by 5)
   - 1: 1:50 dilution (multiply by 50)
   - 2: 1:500 dilution (multiply by 500)
   - 3: 1:5,000 dilution (multiply by 5,000)

**MacConkey plate:**

1. Decide which MacConkey plate (0, 1, 2, or 3) has 25-250 total colonies. 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 all dilutions, the sample will have to be further diluted and re-plated. Ask Joanna for instructions.)

2. Identify and count the colonies as follows.

   - ***Coliforms:*** pink, opaque colonies
     - ***Klebsiella:*** mucoid coliforms with a cream-colored center (report the percentage of coliform colonies which are Klebsiella)

   - ***Non-coliform gram-negatives:*** translucent colonies

3. Multiply the count of each type of colony by the dilution factor of the MacConkey plate. The dilution factors of each plate are as listed above for the Factor plate.