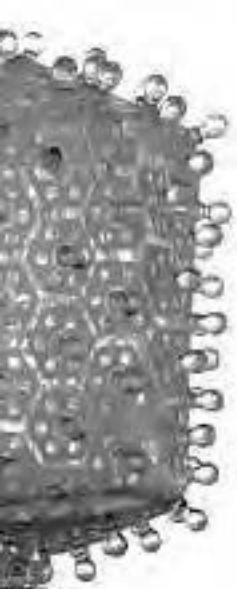

Microbiology

LAB # : 2

Mrym Ghuloom

ISOLATION OF BACTERIA IN PURE CULTURE

- Pure cultures are essential to the accurate determination of colony characteristics, biochemical properties, morphology, staining reaction, immunologic reactions, and susceptibility to antimicrobial agents.

- Microorganisms are ubiquitous; therefore, aseptic techniques must be used during collection of specimens and work with culture media etc.

- The streak-plate method, if properly performed, is probably the most practical and most useful for obtaining discrete colonies and pure cultures. The streak-plate method consists of the spreading of bacterial suspensions over an agar surface in a definite pattern to separate single cells or small clumps of cells from the culture so that isolated colonies will grow during incubation.

MATERIAL

A mixture of broth cultures of *staph.epidermidis* and *Esch.coli*
1 CLED agar plate + 1 Blood agar plate.

PROCEDURE:

A) Inoculate and streak (figure1) one full loop of broth culture on CLED agar and blood agar and incubate it at 37C.

B) At the next day, examine your streak plate and look for well isolate colonies of both species.

C) At the same time prepare a gram-stained smear for microscopic examination.

Transfer to Agar Plates (Quadrant streak Plate)

1. Suspend the organisms in the broth culture or use directly from a slant. Flame the wire loop until it is red. Remove the cap from the culture tube and flame the mouth of the tube. Do not contaminate the cap or loop during this procedure. Remove a loopful of organisms. Flame the mouth again and replace the cap on the tube.

2. Spread the organism over a small region on the edge of the plate as in 1 in the diagram below.

3. Flame the loop and let it cool for a few seconds.

4. Streak from the end of region 1 across the edge of the plate forming region 2.

5. Flame the loop and let it cool for a few seconds.

6. Streak from the end of region 2 across a quarter of the plate forming region 3.

7. Flame the loop and let it cool for a few seconds.

8. Streak from region 3 across the remaining portion of the plate forming region 4.

9. Flame the loop before setting down.

10. Incubate the plate for 24-48 hours in inverted position.

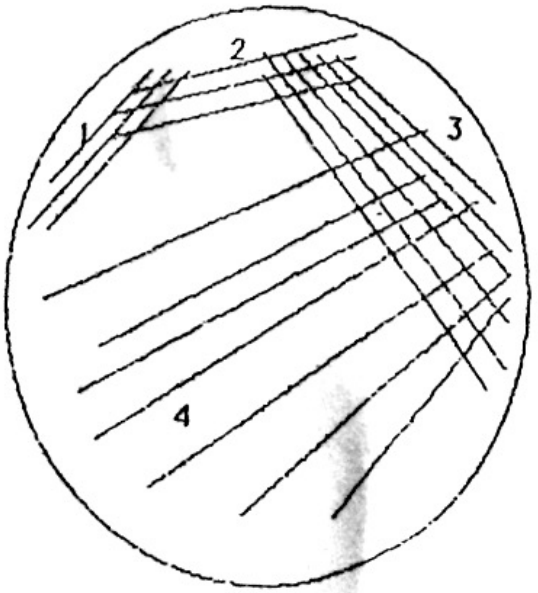

Figure 1

Streak Plate Technique

1. Streak swab of clinical sample over one quarter of the sterile Petri dish. This is Quadrant #1.

2. Discard swab in biohazard bag.

3. Sterilize loop in flame of Bunsen burner.

4. Allow loop to cool without waving it about

5. Place loop on next quadrant of Petri dish, next to Quadrant #1. Gently drag the loop into Quadrant #1 a few times, to obtain just a bit of bacteria from that first sample, then spread that material over Quadrant #2.

6. Again sterilize loop in flame of Bunsen burner, and allow loop to cool without waving it about.

7. Place loop in next quadrant of Petri dish, adjacenty to Quadrant #2. Gently drag the loop into Quadrant #2 a few times, to obtain just a bit of bacteria from that sample, then spread that material over Quadrant #3.

8. Again sterilize loop in flame of Bunsen burner, and allow loop to cool without waving it about.

9. Place loop in next quadrant of Petri dish, adjacent to Quadrant #3. Gently drag the loop into Quadrant #3 a few times, to obtain just a bit of bacteria from that sample, then spread that material over Quadrant #4.

10. Incubate plate at 37 degrees C for at least 24 hours