# II. LABORATORY SESSIONS

# 1ST SEMESTER 2013/2014

| ITEM | TOPIC |
|---|---|
| 1. | Introduction-Safety Rules-Simple Stains- gram Stain & Wet Mount Preparation. |
| 2. | Cultivation Of Bacteria, Collection Of Clinical Specimens (Throat, Sputum, Blood,CSF,Urine) |
| 3. | Antimicrobial Susceptibility Testing. |
| 4. | Staphylococcus,Streptococcus,Bacillus,Diphtheroids, & Neisseriae |
| 5. | E.coli,Pseudomonas,Klebsiella,&Proteus. |

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**GOODLUCK.**

## 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 suseptibility to antimicrobial agents.

Microorganisms are ubiquitous; therefore, aseptic techniques must be used during collection of specimens and work withculture 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 a 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

## MATERIALS

A mixture of broth cultures of Staph.albus and Esch.coli
1 CLED agar plate

## PROCEDURE:

Watch how culture aseptic techniques and streak-plate technique will be illustrated in the lab
1st day:

A) CLED agar plate will be used in the first period. Streak the plate and incubate it.
B) At the next lab period, examine your streak plate and look for well isolated colonies of both species.
C) At the next lab. period prepare a gram-stained smear for microscopic examination. Did you isolate a pure culture? (Staph. or E.coli).

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## 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.

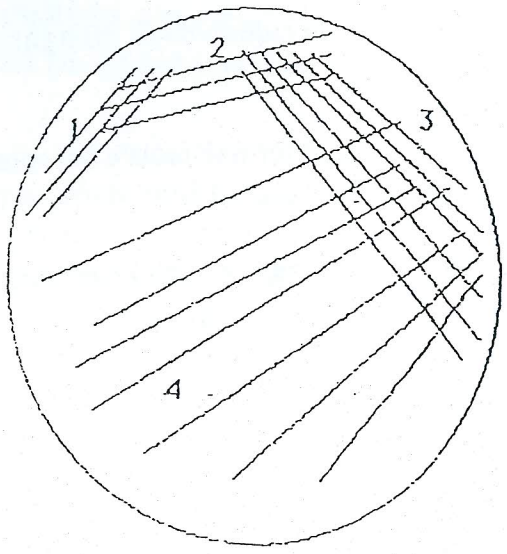


Figure 1