PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Bacterial Culture Inoculation Procedure

Department of Microbiology

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1.0 Purpose

The inoculation of clinical specimens onto culture media is one of the most important procedures in Microbiology. The use of proper media and good technique is essential in the recovery of microorganisms. After a specimen has been inoculated to the appropriate media a series of dilution streaks are made. Streaking the agar surface is achieved by dragging a sterile inoculating loop back-and-forth across the agar. The purpose of this process is to dilute the inoculum sufficiently on the surface of the agar medium so that well-isolated colonies of bacteria, known as colony-forming units (CFU) can be obtained.

2.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

This procedure may expose you to:

- Airborne, bloodborne, and enteric pathogens

To perform this procedure, you must use:

- Gloves
- Laboratory Coat
- Biological safety cabinet (for specimen processing)

Disinfectant following procedure:

- Use bleach dilution sprayers or disinfectant wipes

Reference for spill/decontamination

- Microbiology Biohazards and Safety

3.0 Materials

3.1 Equipment and/or Testing System

- Incinerator

3.2 Consumables

- Sterile swabs
- Inoculation loops (use calibrated loops for urine specimens)

4.0 Procedure

4.1 Urine Specimens

1. Urine is streaked onto plates using either the 1:100 mL (10 μL) calibrated inoculating loop or the 1:1000 (1 μL) calibrated inoculating loop, depending on the method used to collect the urine.
2. To inoculate a urine culture, use the appropriate calibrated inoculating loop. Mix the specimen prior to sampling. Dip the loop straight into the urine. Make one streak down the blood agar side of the plate (see Fig. 1), and proceed to cross streak making very close lines (see Fig. 2). Using the same loop, dip straight into the urine again, and inoculate and streak the CHROMagar side of the biplate (Fig. 3). If necessary, a smear can be made last using the 1:100 calibrated loop.

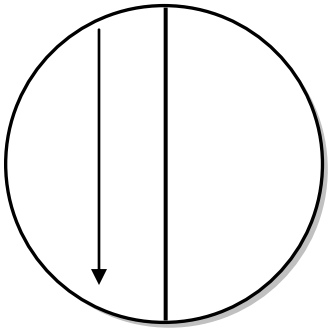


Fig. 1

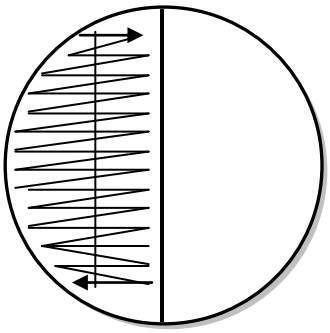


Fig. 2

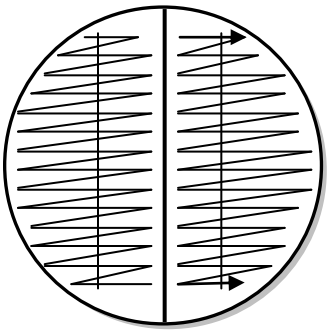


Fig. 3

4.2 Body Fluid Specimens

1. Centrifuge fluid according to guidelines in the Specimen Processing procedure for Body Fluids. Do not centrifuge bloody or purulent fluids.
2. A sterile pipette may be used to transfer a drop of fluid to the 1st quadrant of each plate (See Fig. 4). Avoid inoculating the material all the way to the edges of the plate.
3. Cross-streak the specimen (Fig. 5) and proceed with streaking for isolation as outlined below in section 4.6.

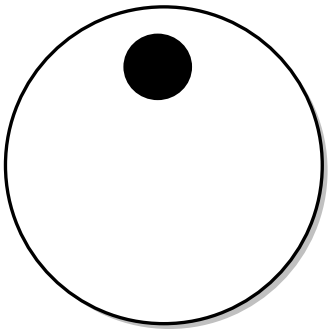


Fig. 4

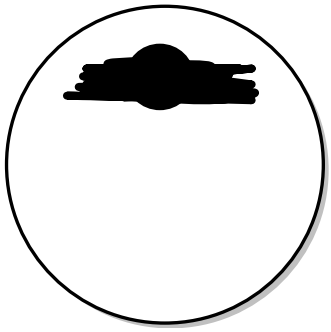


Fig. 5

4.3 Wound Specimens & Specimens Received on Swabs in Transport Medium

1. Make a smear first by rolling the swab in a quarter-size area in the middle of a glass slide.
2. Starting with non-selective media (i.e. blood and chocolate agar), inoculate each plate in the first quadrant by rolling the swab so that its entire surface comes into contact with the plate. Inoculate the first quadrant of each plate (see Fig. 6 below).
3. Return the swab to the transport medium.
4. Streak for isolation as outlined below in section 4.6.

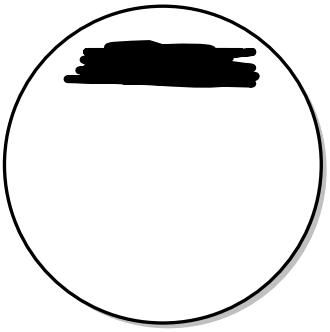


Fig. 6

4.4 Lower Respiratory Specimens

Select portions that appear purulent and, if possible, avoid clear/foamy areas that resemble saliva.

1. Using a sterile swab, transfer material to plates by inoculating the first quadrant of each plate (see Fig. 6 above).
2. Prepare a smear by spreading the material over a quarter-size area in the middle of a glass slide.
3. Streak for isolation as outlined below in section 4.6.

4.5 Stool Specimens

1. Using a sterile swab, transfer material to plates by inoculating the first quadrant of each plate (Fig. 6 above).
2. Prepare a smear, if required, by spreading the material over a quarter-size area in the middle of a glass slide. Material should be thin enough to read newsprint through.
3. Streak for isolation as outlined below in section 4.6.

4.6 Streaking for Isolation

Use a sterile inoculating loop to streak 2nd, 3rd, and 4th quadrants (See Fig. 7, 8, and 9). With each new quadrant, begin by dragging the inoculating loop through the previous quadrant 2-4 times. **Be sure to sterilize the inoculating loop in the incinerator in between quadrants**. At a minimum, the loop may be flipped so that a sterile edge is used one time for each quadrant. **Always sterilize the inoculating loop between plates**.

Fig. 7

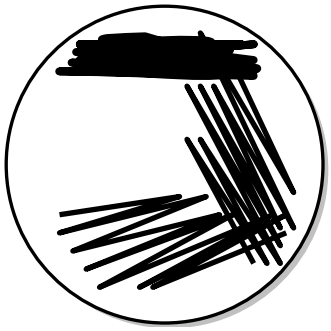


Fig. 8

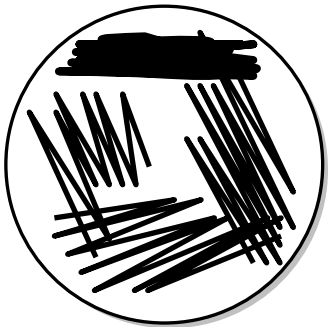


Fig. 9

*When streaking the CHOC/MTM split plate for genital cultures inoculate specimen by rolling the swab in a quarter-size area at one end of the plate on both sides. Streak at least two additional quadrants on each side to help ensure colony isolation (see Fig. 10).

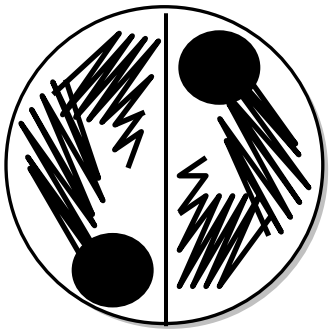
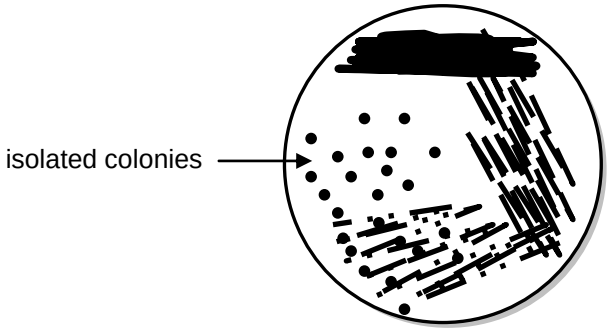


Fig. 10

5.0 Interpretation

Effective specimen streaking will yield isolated colonies after incubation. The number of quadrants that contain growth will depend on the concentration of bacteria in the original specimen.



6.0 Limitations

In the process of handling specimens in the laboratory, extraneous organisms may be introduced from the environment or from the indigenous flora of the individuals that handle the culture. Working within a Class II biologic safety cabinet while handling the specimen and culture media helps to prevent such contamination.

7.0 Competency Assessment

Each person that works in the specimen processing area must demonstrate proper streaking technique. A heavy suspension of mixed ATCC organisms is used as a test inoculum. Evaluation and feedback will be provided for technique improvement if necessary.

8.0 References

Winn, W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., Woods, G. (2006). Phases of the Diagnostic Cycle in: *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th edition., Philadelphia: Lippincott, pp. 27-33.

9.0 Document Control History

Adopted/Approved by Microbiology director (AR) 08/03/2007

Approved by medical director J. Schappert 03/10/2010

Reviewed by supervisor (JC) 08/03/2007, 11/2008, 11/2009, 5/2011, 3/2012, 3/2014