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.

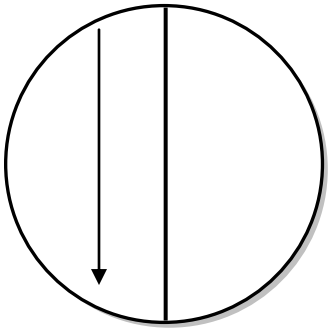


Fig. 1

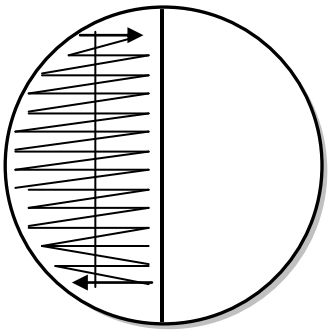


Fig. 2

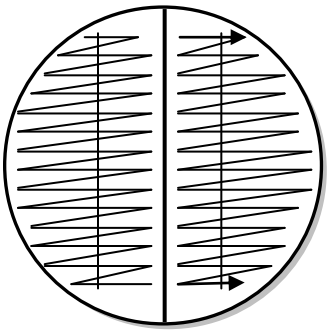


Fig. 3

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.

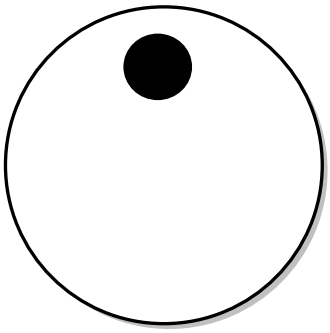


Fig. 4

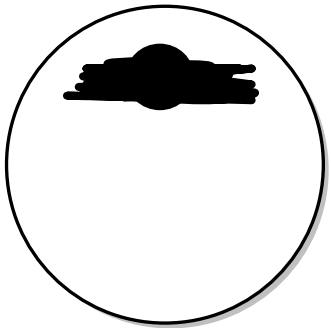


Fig. 5

4.3 Wound Specimens & Specimens Received on Swabs in Transport Medium

4.3.1 Spun-fiber Swabs

Smear

1. Prepare smear for Gram stain by pressing and rolling the swab in the center of a glass slide to express absorbed material.
2. Fix smears using methanol for 1 min and allow the smear to air dry. Methanol fixation is recommended as it prevents lysis of red blood cells, avoids damage to all host cells, results in a cleaner background, and prevents liquid specimens from washing off.

Culture

1. Use the swab to directly inoculate the media. Press and roll the swab against the surface of the agar in the first quadrant (see Fig. 6 below). Inoculate the least inhibitory media first (BAP & CHOC).
2. Quadrant-streak each plate for isolation (see section 4.6).

4.3.2 Flocked Swabs in Liquid Amies (ESwab)

1. Vigorously shake the ESwab tube containing the swab sample between the thumb and forefinger for 5 s or mix the tube using a vortex for 5 s to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid transport medium.
2. Unscrew the ESwab cap and remove the swab applicator.

Smear

1. Prepare a smear for Gram stain by transferring 1 or 2 drops of the suspension onto the center of a glass microscope slide, using either the swab or a transfer pipette. In case of bloody or thicker specimens, care should be taken to thinly spread the sample on the slide. Place the slide in an electric slide warmer set at a temperature not exceeding 42°C.
2. Fix smears using methanol for 1 min and allow the smear to air dry. Methanol fixation is recommended as it prevents lysis of red blood cells, avoids damage to all host cells, results in a cleaner background, and prevents liquid specimens from washing off.

Culture

1. Roll the tip of the ESwab applicator onto the surface of the first quadrant of the culture plate to provide the primary inoculum (see Fig. 6 below).
2. If it is necessary to inoculate additional media, return the ESwab applicator to the transport medium tube for 2 s to absorb and recharge the applicator tip before inoculating each additional plate. Alternatively, a sterile pipette can be used to transfer 1 to 3 drops of the suspension onto each culture plate.
3. Quadrant-streak each plate for isolation (see section 4.6).

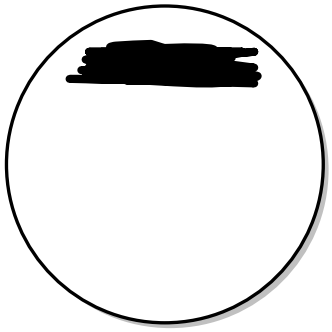


Fig. 6

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

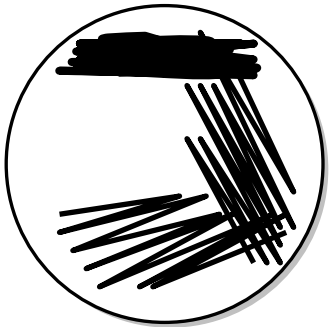


Fig. 8

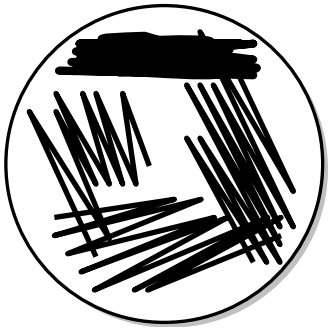
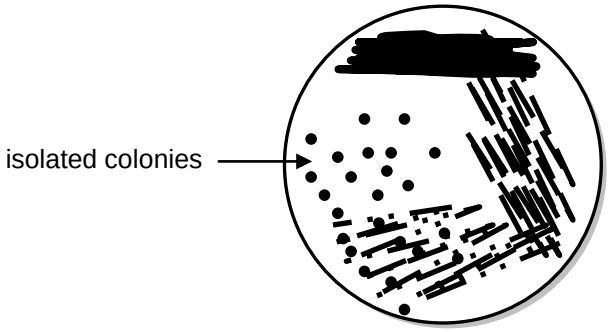


Fig. 9

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

1. 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.
2. Package insert: Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System, Rev. 04, 10/2010.

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

Revisions & Updates: 12/30/2014 Updated procedure for ESwab and removed MTM/CHOC split plate streaking.