Inoculation and Streaking of Culture

PURPOSE

To serve as a guide when performing primary inoculation of clinical specimens. The initial handling of specimens for Bacteriology is a multifaceted procedure. The specimens must be matched to the requisition to make sure there are no discrepancies. The determination also must be made as to what type of category the specimen falls into to determine how and what to plate the specimen on.

PRINCIPLE

A biological safety cabinet should be used during the manual processing of all specimens

- All specimens should be plated as soon as possible upon receipt
- The source of each specimen along with the date and time of collection should be specified. This information can be found by accessing the Laboratory Information System (LIS) either on Cerner or information located on the patient barcode labels.
- All primary bacteriology specimens should be processed, inoculated, and incubated per techniques described in this procedure
- Each specimen received must be accompanied by the appropriate physician order
- All specimens must be labeled with the patient's name, medical record number, collection date and time, and order type

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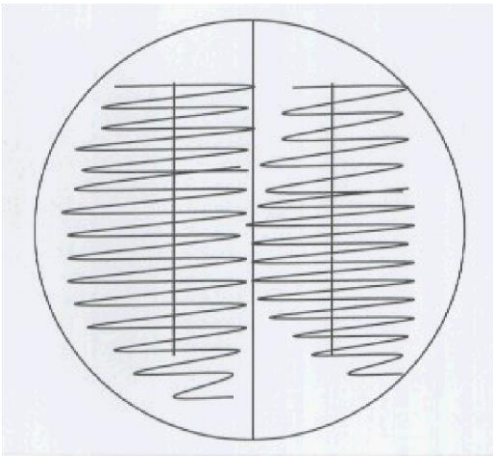
Safety or Special Safety Precautions

When preparing specimens for culture analysis, observe universal precautions always. Whenever possible, all steps required in the processing of specimens should be done in a laminar-flow biological safety hood.

PROCEDURE

Urine Specimens	
Step	**Action**
1	Mix specimen thoroughly by swirling urine cup.
	Depending on the order use either a calibrated loop 0.001 or larger 0.01 loop.
2	Dip the calibrated loop into the urine specimen.
3	Streak down the center of one side of the biplate Then streak the entire surface with perpendicular lines.
4	Dip the tip of the loop into the urine specimen.
5	Streak down the center of the other side of the biplate Then streak the entire surface with perpendicular lines. (Refer to Figure 1.)  Figure 1

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PROCEDURE **Swab Collected Specimens:**

For any specimen collected with a swab and submitted in transport media, the inoculation and streaking will be done by the four-quadrant method. The plates used will vary according to the source. (Refer to the plating chart)

Swab Collected Specimens	
Step	**Action**
1	Select the appropriate plates for the source indicated.
2	Vigorously shake the eSwab© tube for about 5 seconds to release and suspend sample into the **liquid** transport media inside tube.
3	Using the attached swab, inoculate each needed media with the liquid transport media from the eSwab© container. Re-wet the swab with transport media in between each plate. If a swab is not included, use a sterile pipette and place 2 drops of the liquid transport media on one quadrant of each plate.
4	Inoculate non-selective media first then inoculate selective media, refer to individual protocol for order.
5	Using a sterile loop, streak inoculated media for isolation as shown in Figure 2.  Figure 2

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PROCEDURE **Respiratory Specimens:**

For Respiratory specimens, the plates used will vary according to the source or test ordered. (Refer to the plating chart)

Respiratory Specimens	
Step	**Action**
1	Select the appropriate plates for the source indicated.
3	Using a sterile swab to mix the sample in the sterile cup, place a sample amount of sample on one quadrant of each plate. Re-wet the swab with the sample in between each plate.
4	Inoculate non-selective media first then inoculate selective media, refer to individual protocol for order.
5	Using a sterile loop, streak inoculated media for isolation as shown in Figure 2.  Figure 2

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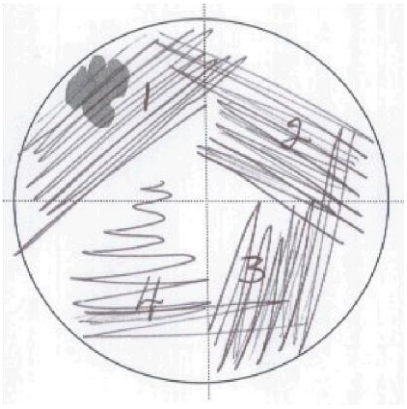
PROCEDURE

Sterile Site/Fluids

There are many types of fluids submitted to the laboratory for culture and organism identification. In this case, the fluid must be placed into a sterile tube, centrifuged and then inoculated onto the appropriate media.

Sterile Site/Fluid Specimens	
Step	**Action**
1	If the fluid was not submitted in a tube, some must be poured off into a sterile plain vacutainer tube. Label the tube with the patient's name, MR#, source of the fluid and the date of collection.
2	Centrifuge the specimen for 3 minutes.
3	Remove the tube from the centrifuge and open it carefully.
4	Discard the supernatant (liquid portion of sample) making sure to leave the cell button at the bottom of the tube.
5	Using a pipette or a swab, collect the cell button and inoculate on the plate. One drop can be used for a gram stain (if cytospin is not available) and the rest for inoculation onto the appropriate plates and/or thioglycolate media.
6	Inoculate the first quadrant of each of the appropriate plates for the source indicated.
7	Using a sterile loop, streak inoculated media for isolation as shown in Figure 2.  Figure 2

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HISTORY PAGE

Type of Change: New	Description of Change(s)	Name of Responsible Person/Date	Operations Director, Area Laboratory Review/Date	CLIA Laboratory Director Review/Date	Date Change Implemented
Major	Regional Template revision Added use of disposable loop Aligned procedure with Regional Lab	Ruby Co 10/25/18			