TRAINING UPDATE

Lab Location:	SGAH & WAH	**Date Distributed:**	4/10/13
Department:	Micro	**Due Date:**	5/15/13

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Specimen Processing for Microbiology SGAH.M04, WAH.M04 v006
Description of change(s):
5.2 Add exception for MRSA chromogenic medium 5.3.3 Add centrifugation of sterile body fluids 5.3.4 Add environmental cultures

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 006)

Non-Technical SOP

Title	**Specimen Processing for Microbiology**	
Prepared by	Ronald Master	Date: 4/14/2009
Owner	Ronald Master	Date: 4/14/2009

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Effective Date:		

Review:		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. PURPOSE

To describe the process for microbiology specimen setup, plating and management.

2. SCOPE

The scope of this SOP is to ensure the pre analytic processes for microbiology specimens are outlined. These procedures are imperative in determining what pathogenic organisms are present in specimens obtained from patients.

3. RESPONSIBILITY

It is the responsibility of all personnel assigned to Microbiology to read, understand and to perform all procedures as described in this SOP.

4. DEFINITIONS

Plating – inoculation of plated/tubes media with clinical specimen for microbiology culture.

Inoculation – to implant microorganisms or infectious material onto a culture medium.

Streaking – The use of a loop or other plating tool to inoculate a specimen in order to differentiate microorganisms by color or texture from its surroundings on a culture medium.

5. PROCEDURE

5.1 Routine Procedure for Plating Cultures:

All specimens are to be plated in a biosafety cabinet.

1. **Media and its location:**

   All routine media will be stored in the refrigerator. Microbiology media should be kept in the refrigerator until needed. Media should be allowed to warm to room temperature before use. A working supply (minimum amount) is left at room temperature for use.

2. **Loops, Swabs, and Pipettes:**

   a. Loops - A wire loop is used for streaking specimens, with the exception of urines. A 0.001 mL calibrated loop must be used to inoculate urine. For sterile urines (cystoscopy, suprapubic aspirate, etc.) use both 0.001 and 0.01 mL calibrated loops.

   b. Swabs - Swabs are used in making the initial inoculation of plates, for preparing smears, and for inoculating specimens into broth media. If a specimen is submitted on a swab it must be submitted in a culturette containing holding medium to prevent drying out. A swab is convenient for inoculating certain specimens onto media, e.g., stool, sputum. Sterile swabs are available at the plating bench.

   c. Pipettes - A sterile pipette may be used to inoculate liquid specimens into broth media, such as thioglycolate, and any plated media. A sterile pipette should be used to inoculate CSF and other body fluids and environmental cultures of liquids. To inoculate thioglycolate with a pipette, insert pipette to bottom of tube and slowly evacuate sample as you with draw the pipette.

3. **Preparation of Smears and Gram Stains:**

   a. Smears - Write the accession number, specimen source, date and the patient's last name on the slide. Using a sterile loop or swab, make a smear about the size of a nickel near the center of the slide. Let the slide air dry, then heat fix. ALWAYS MAKE SMEAR AFTER INOCULATING MEDIA TO AVOID CONTAMINATING THE SPECIMEN.

4. **Inoculation and Streaking of Media:**

   a. The first process in the cultural examination of clinical specimens is the selection of appropriate isolation media. Addenda A, Figure 3 lists the media suitable for the isolation of microorganisms most commonly recovered from various clinical specimens. It is desirable to inoculate more than one kind of isolation medium.

   b. The purpose of isolation is to obtain bacterial colonies representing progeny of a single cell and thus provide the source of a pure culture. The streaking of materials onto the surface of the medium provides such results. The streaking method must be such that, (a) part of the medium is inoculated with a large amount of material, and (b) subsequent streaking will allow for growth of isolated colonies. When done properly, the completed streaking should cover essentially

the entire surface of the medium. When streaking plates, flame the loop between the first and second streak area to avoid overly heavy growth or use a disposable loop. An illustration and explanation of an acceptable method is provided in Addendum A, Figure 1. Addendum A, Figure 2 illustrates the proper streaking procedure for a urine colony count.

5.2 Incubation of Plates:

Plates and broth media are incubated in a CO_2 incubator at 35 ±2°C except for chromogenic medium for MRSA which is incubated at 35-37°C in air (non-CO_2).

5.3 Specimens and Special Requirements:

5.3.1 Stool Cultures

1. If *E. coli* O157 is ordered, use test code XECOL.
2. Stool in transport media is to be sent to Chantilly. Stool specimens will be plated in Chantilly.

5.3.2 IV Catheter Tips

1. Perform all steps in a biological safety cabinet.
2. Using sterile forceps, remove catheter tip from transport tube.
3. Lay the catheter tip on a blood agar plate, and using sterile forceps, roll tip 4-5 times over entire plate. If the catheter tip is longer than 2 inches (5 cm), use sterile scissors or scalpel to cut the end closest to the top of the tube (proximal end) prior to rolling the distal end on the plate. The proximal end may be rolled on a second plate, if desired.
4. Leave the catheter tip on the plate, do not press it into the agar.

5.3.3 Cerebrospinal Fluid and Other Sterile Body Fluids

1. Sterile body fluids must be concentrated by centrifugation prior to inoculation of media.
2. If ≥ 1 mL of fluid is submitted for culture, centrifuge at 1500xg for 15 minutes.
3. Use the sediment to inoculate the media.
4. If < 1 mL of fluid is received, add the comment "Culture results may be compromised by the limited volume of specimen received."

5.3.4 Environmental Specimens

1. Environmental samples are specimens other than from patients.
2. These specimens are primarily ordered by Infection Control and Prevention, Pharmacy, the Laboratory and Dialysis. The most common samples received are listed below. For other requests contact a supervisor or Chantilly microbiology.
3. Laboratory
   a. Water used for laboratory reagents
   b. 1 mL of lab water is inoculated onto a Standard Methods agar plate
   c. Use a sterile loop to spread the inoculum evenly over the entire plate
   d. Leave the plate with the lid up until the water has been absorbed into the medium
   e. Accession, label and transport to Chantilly

4. Dialysis
   a. Dialysis water and dialysate
   b. 1 mL of lab water is inoculated onto a Trypticase soy agar (TSA) plate
   c. Use a sterile loop to spread the inoculum evenly over the entire plate
   d. Leave the plate with the lid up until the water has been absorbed into the medium
   e. Accession, label and transport to Chantilly
5. Pharmacy
   a. Media Fill Test or Aseptic Test Kit
      i. Order as XENVR, Specimen description ENVIR -; MEDIA FILL
      ii. The mini-bags that are submitted have been inoculated by the pharmacy personnel
      iii. Accession, label and transport to Chantilly
   b. Gloved finger samples
      i. Order as XENVR, Specimen description ENVIR -; GLOVE
      ii. 2 Trypticase soy agar (TSA) plates will be submitted per employee and have been inoculated
      iii. Do NOT cross streak these plates
      iv. Accession, label and transport to Chantilly
   c. Settle Plates from Hoods
      i. Order as XENVR, Specimen description ENVIR -; HOOD
      ii. Both a Trypticase soy agar (TSA) plates and a Sabouraud agar (SAB) plate will submitted for each hood. They have been inoculated
      iii. Do NOT cross streak these plates
      iv. Accession, label and transport to Chantilly
   d. Surface Cultures
      i. Order as XENVR, Specimen description ENVIR -; SURFACE
      ii. A single swab will be submitted for each culture
      iii. Inoculate the swab into a tube of Trypticase soy broth (TSB). Break the shaft of the swab off leaving the swab in the broth. If TSB is not available, thioglycolate broth may be used.
      iv. Accession, label and transport to Chantilly
6. Infection Control and Prevention
   a. Most samples will be swabs from surfaces.
   b. Order as XENVR and add the appropriate specimen description
   c. Inoculate the swab onto the first quadrant of a Sheep blood agar plate (BAP)
   d. Use a sterile loop to streak in 4 quadrants to obtain isolated colonies

6. RELATED DOCUMENTS

N/A

7. REFERENCES

N/A

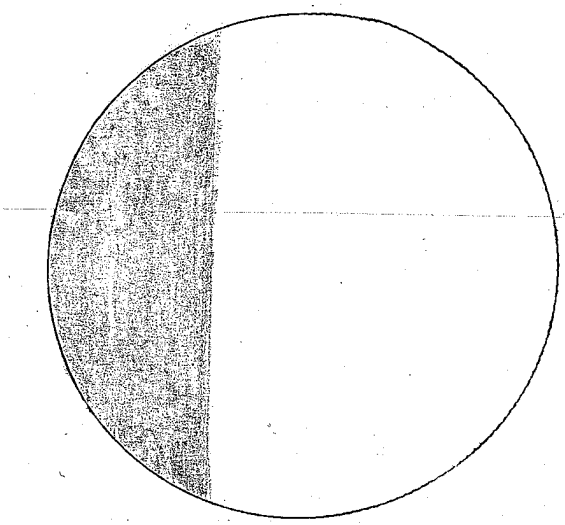
8. REVISION HISTORY

Version	Date	Reason for Revision	Revised By	Approved By
		Supersedes SOP M006.007		
000	5/26/09	Addenda D: media change for MRSA screen	L. Barrett	R. Master
001	3/10/10	Section 5: Change stool culture, add IV cath tip	R. Master	R. Master
002	5/17/10	Section 5.1: Delete requirement for date on opened media 5.2: Change temperature to 35 ± 2°C	R. Master	R. Master
003	7/12/11	5.3.2 Specified catheter length	R. Master	R. Master
004	5/21/12	5.3.1 Deleted plated media for stool cultures	R. Master	R. Master
004	5/21/12	Figure 4: Updated stool cultures	R. Master	R. Master
005	4/9/13	5.2 Add exception for MRSA chromogenic medium 5.3.3 Add centrifugation of sterile body fluids 5.3.4 Add environmental cultures	R. Master	R. Master

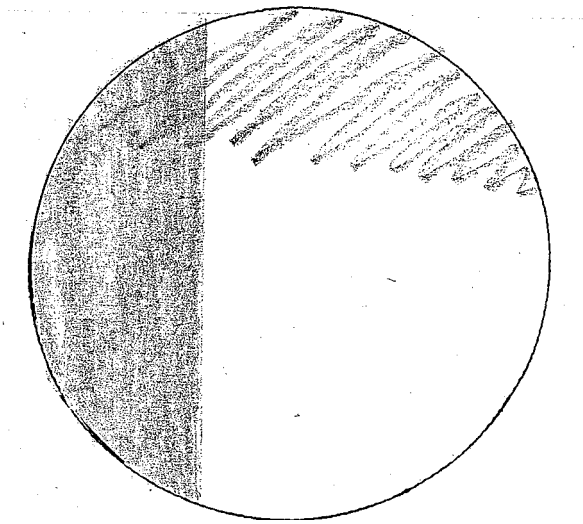
9. ADDENDA

A. Figure 1 – One Acceptable Method of Plate Streaking
B. Figure 2 – Proper Streaking for a Urine Culture and Colony Count
C. Figure 3 – Proper Streaking for a Biplate
D. Figure 4 – Plating Chart for Media by Source and Test Code (see Attachment tab of Infocard)

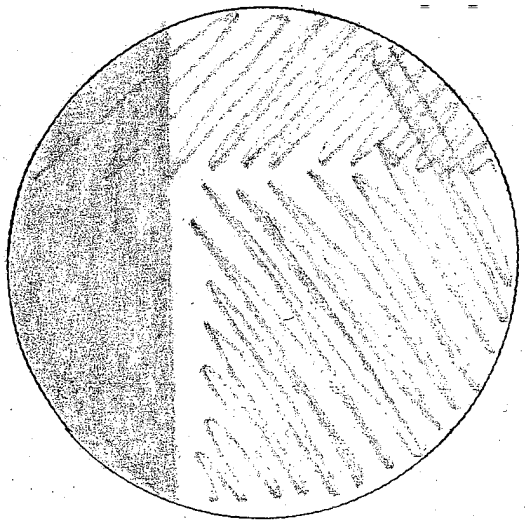
Figure1 **<u>One Acceptable Method of Plate Streaking</u>**



Step 1: Using a loop or a swab inoculate the specimen onto one edge of the plate, covering about one third of the plate.

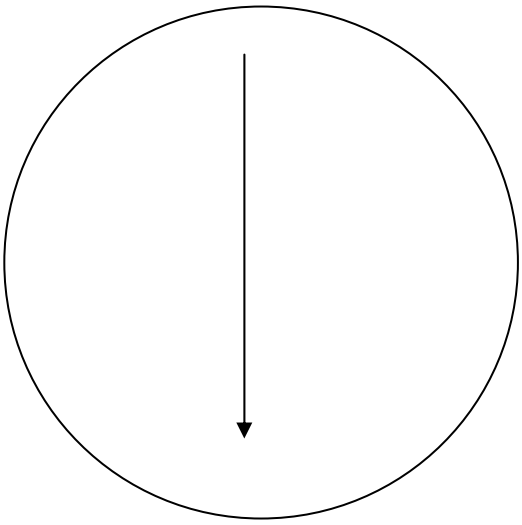


Step 2: Flame loop arid cool it by stabbing into the sterile agar. With cooled loop, streak at a right angle to the initial inoculum going back and forth many times.

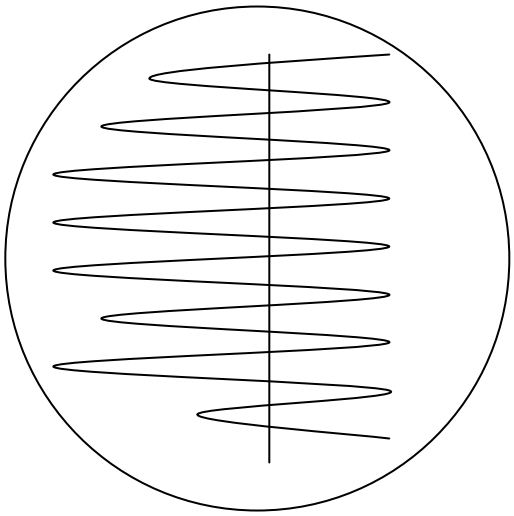


Step 3: Flame loop if specimen is likely to contain a lot of normal flora. Cool the loop. Rotate plate again, and entering only the isolation area, draw loop over the-previously uninoculated portion of the plate. (Be careful to not streak over the initial inoculum.)

Figure 2 **Proper Streaking for a Urine Culture and Colony Count**

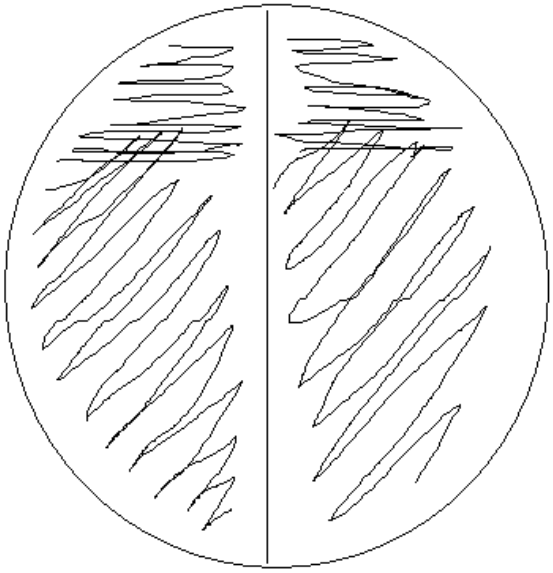


Step 1: Swirl urine to mix. Select sterile calibrated loop. Dip into the bottom of the urine sample and streak down the middle of the plate.



Step 2: Starting at the top, go back and forth numerous times over the initial streak line to facilitate the isolation of bacterial colonies.

Figure 3 **Proper Streaking of Bi-plates (non-urine specimens)**



Biplates are inoculated by initially streaking about 15-20% of the plate, then flaming before performing the downward streak.