TRAINING UPDATE

Lab Location:	SGAH & WAH	**Date Distributed:**	5/11/2015
Department:	Micro	**Due Date:**	6/2/2015
		Implementation:	**6/3/2015**

DESCRIPTION OF REVISION

Name of procedure:

Specimen Processing for Microbiology SGAH / WAH.M04 v7

Sources for Anaerobic Culture Table AG.F326.0

Description of change(s):

SOP -

Section 5.3.3 Edited centrifugation of sterile body fluids (*to match actual practice*)

Section 6: Moved plating chart from section 9, added Anaerobic culture table

No changes to Sources for Anaerobic Culture Table, just placed under document control

This SOP and FORM will be implemented on June 3, 2015

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

Approved draft for training (version 7)

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 processed immediately upon receipt.
2. Include a chocolate agar plate and a thioglycollate broth for all sterile body fluids submitted for culture.

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

Plating Chart for Media by Source and Test Code (AG.F191)
Sources for Anaerobic Culture Table (AG.F326)

7. REFERENCES

N/A

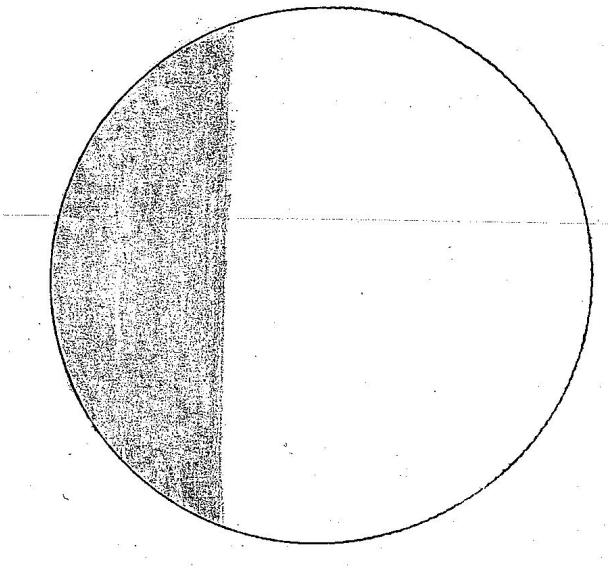
8. REVISION HISTORY

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
006	4/27/15	5.3.3 Edited centrifugation of sterile body fluids Section 6: Moved plating chart from section 9, added Anaerobic culture table Footer: Version # leading zero's dropped due to new EDCS in use as of 10/7/13	R. Master	R. Master

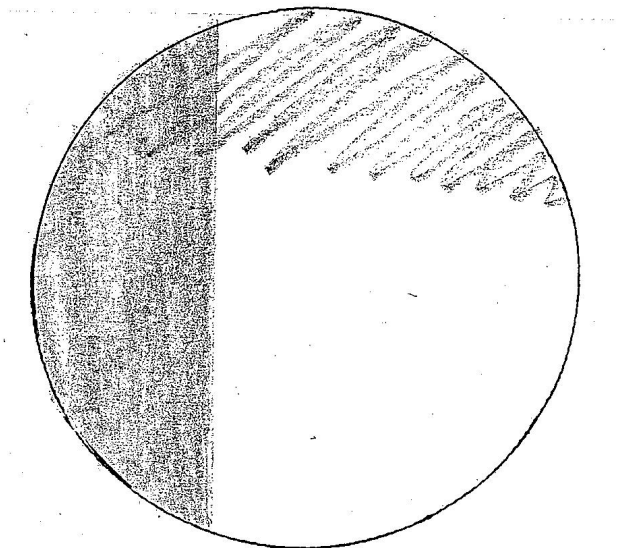
9. ADDENDA

A. Figure 1 – 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)~~

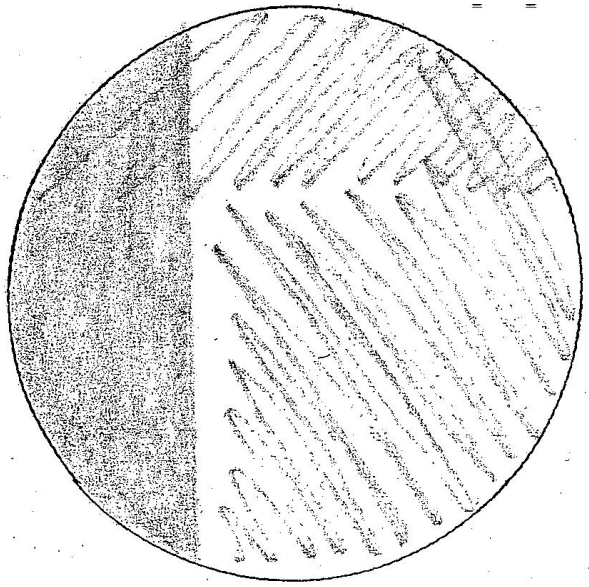
Figure 1 **Acceptable Method of Plate Streaking**



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

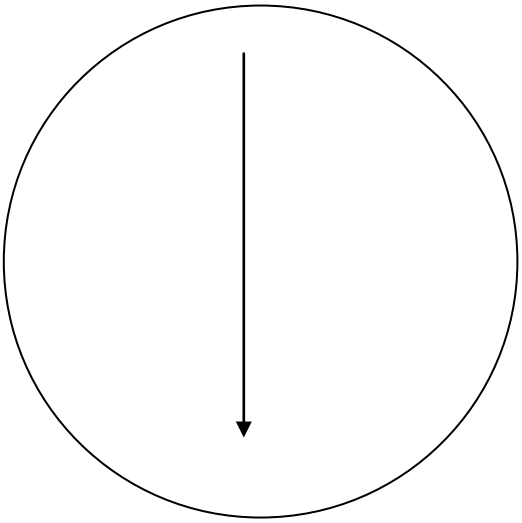


Step 2: Flame loop arid cool it by stabbing into the sterile agar. With cooled loop, streak at a right angle to the initial inoculum going back and forth many times.

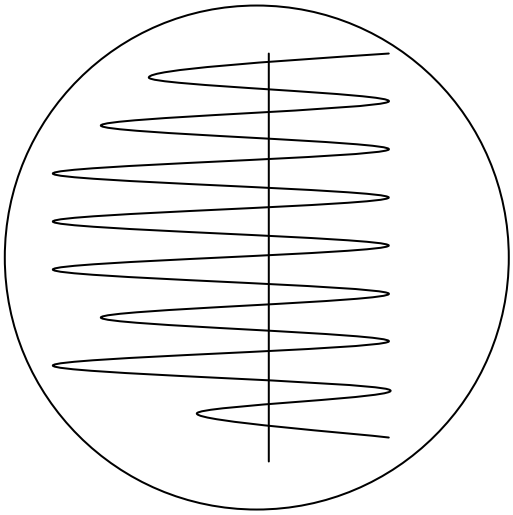


Step 3: Flame loop if specimen is likely to contain a lot of normal flora. Cool the loop. Rotate plate again, and entering only the isolation area, draw loop over the-previously uninoculated portion of the plate. (Be careful to not streak over the initial inoculum.)

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

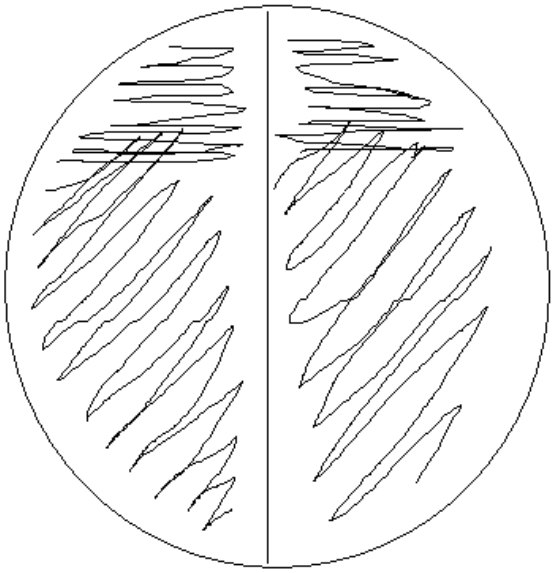


Step 1: Swirl urine to mix. Select sterile calibrated loop. Dip into the bottom of the urine sample and streak down the middle of the plate.



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.

Body Fluid Cultures

* The following specimen sources must have both a Fluid Culture (XFLC) and an Anaerobe Culture (XANAC) ordered

Acceptable Specimens for Body Fluid Culture	Order	XANAC (always order)
abdominal fluid	XFLC + XANAC	yes
amniotic fluid	XFLC + XANAC	yes
ascitic fluid	XFLC + XANAC	yes
bile	XFLC + XANAC	yes
gallbladder fluid	XFLC + XANAC	yes
joint fluid	XFLC + XANAC	yes
paracentesis fluid	XFLC + XANAC	yes
pericardial fluid	XFLC + XANAC	yes
peritoneal fluid	XFLC + XANAC	yes
pleural fluid	XFLC + XANAC	yes
synovial fluid	XFLC + XANAC	yes
thoracentesis fluid	XFLC + XANAC	yes

Prosthetic Joint Culture	Order	XANAC
prothesis	XJOINT+XANAC	yes
synovial fluid (if prosthetic joint present)	XJOINT+XANAC	yes
tissue (if prosthetic joint present)	XJOINT+XANAC	yes

The following specimen sources may **NOT** be ordered as a Fluid Culture (XFLC)

CSF Culture	Order	XANAC
CSF	XCSFC	no

Respiratory Culture	Order	XANAC
bronchial alveolar lavage (BAL)	XRESP	no
bronchial washings	XRESP	no
endotracheal aspirate	XRESP	no
tracheal aspirate	XRESP	no
transtracheal asirate	XRESP	no
sputum	XRESP	no
any other respiratory source other than throat	XRESP	no
throat	XTC	no
nasopharynx (NP)	XRESP	no
nose	XRESP	no
oral	XRESP	no
sinus aspirate	XRESP	if specifically ordered

The following specimen sources may **NOT** be ordered as a Fluid Culture (XFLC)

Source	Order
CSF	XCSFC
any respiratory source	XRESP
any wound	XWDCA
any urinary source	XURNC
blood	XBLC
bone marrow	XBLC
tissue from skin surface	XTISC or XSURG
deep tissue	XSURG
abscess	XWDCA
any genital source	XGENC

Ear Culture	**Order**	**XANAC**
ear	XEAR	no
tympanocentesis	XEAR or XSURG	yes

Eye Culture	**Order**	**XANAC**
eye (source should be more specific)	XEYE	no
conjunctiva	XEYE	no
cornea	XEYE	no
ocular fluid / intraocular fluid	XSURG or XEYE	if specifically ordered
anterior chamber fluid	XSURG or XEYE	if specifically ordered
vitreous fluid	XSURG or XEYE	if specifically ordered

Wound Culture	**Order**	**XANAC**
abscess	XWDCA	if specifically ordered
bite	XWDCA	if specifically ordered
burn	XWDCA	no
cellulitis	XWDCA	no
cyst	XWDCA	no
decubitis	XWDCA	no
exudate	XWDCA	no
hematoma	XWDCA	no
laceration	XWDCA	no
lesion	XWDCA	no
pus	XWDCA	no
skin	XWDCA	no
stoma	XWDCA	no
surgical wound	XWDCA	if specifically ordered
ulcer	XWDCA	no
vesicle	XWDCA	no
wound aspirate	XWDCA	if specifically ordered
wound drainage	XWDCA	no

Urine Culture	**Order**	**XANAC**
suprapubic aspirate	XURNC	if specifically ordered
any other urinary source	XURNC	no

* Do NOT order any urine specimen as a surgical culture

Blood Culture (NOT for fungus or AFB)	Order	XANAC (do not order)
blood	XBLC	Included
bone marrow	XBLC	Included

Tissue or Surgical Culture (use tissue if tissue is submitted, use surgical if submitted on a swab as a surgical culture)	Order	XANAC
aspirate	XTISC or XSURG	if specifically ordered
biopsy	XTISC or XSURG	if specifically ordered
bite	XTISC or XSURG	if specifically ordered
bone	XTISC or XSURG	if specifically ordered
brain	XTISC or XSURG	if specifically ordered
burn	XTISC or XSURG	no
deep tissue	XTISC or XSURG	if specifically ordered
heart valve	XTISC or XSURG	if specifically ordered
liver	XTISC or XSURG	if specifically ordered
lung	XTISC or XSURG	if specifically ordered
tissue from skin surface	XTISC or XSURG	no

Genital Culture	Order	XANAC
placenta (cesarean section)	XGENC	if specifically ordered
placenta (vaginal birth)	XGENC	no
endometrium	XGENC	if specifically ordered
uterus	XGENC	if specifically ordered
culdocentesis	XGENC	if specifically ordered
fallopian tube	XGENC	if specifically ordered
ovary	XGENC	if specifically ordered
bartholin's gland	XGENC	if specifically ordered
cervix	XGENC	no
endocervix	XGENC	no
vagina	XGENC	no
urethra	XGENC	no
vulva	XGENC	no
lochia	XGENC	no

PLAC-; maternal
PLAC-; fetal

4/27/2015