Miscellaneous Culture

Purpose

This procedure provides instructions for Miscellaneous Culture for the Microbiology laboratory.

Principal and Clinical Significance

Shunt tubing and other specimen types, such as epilepsy grids, port-a-cath hubs, heart patches and other hardware devices put the patient at significant risk for device-related infection. The implanted hardware becomes colonized by bacteria from the patient's own skin and mucous membranes or by microorganisms carried on the hands of medical personnel.

Policy Statements

This procedure applies to Microbiologists who perform culture set-up and plate reading.

Test Code

MMC

Materials

	Reagents	Supplies	Equipment	Media
	• Gram Stain reagents	• Glass slide (GMST) • Anaerobic Gas Pack • Sterile disposable pipette • Sterile container/tube • Palladox catalysts • Inoculating loop	• Ambient air incubator • Anaerobic jar • CO_2 incubator • Incinerator • Microscope • Vortex mixer • Anoxomat	Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media. • Chocolate agar (CHOC) • Sheep Blood agar (SB) • CNA agar (CNA) • MacConkey agar (MAC) • Thioglycolate (THIO) • Saline, Normal 1.0 mL (SLNE)

Specimen

A. Acceptable specimens
- Specimens that are not standard culture types (not AC, BF, SKIC, TISC or WDC)
- Shunt tubing
- Heart patch
- Port-a-cath hub
- Epilepsy grid
- Tracheostomy tubing
- Medical devices

B. Special instructions
- Culture within 2 hours of receipt in lab
- State specific site of specimen
- All medical devices should be brought to the Histology lab after processing, for storage. Examples include:
  - Pacemakers
  - Vagal nerve stimulator
  - Bladder stimulator (sacral nerve neuromodulator)
  - Prosthetic joints
  - Metal heart valves
- Tubing, catheters, shunts, etc. do not need to be brought to Histology.

C. Refer to the Lab Test Directory for Specimen Collection and Transport: - Miscellaneous Culture and Gram Stain

Special Safety Precautions

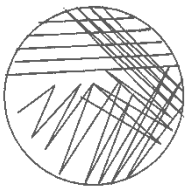
Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**

- *Biohazard Containment*
- *Biohazardous Spills*
- *Safety in the Microbiology Laboratory*

Procedure

A. **Inoculation**
1. Allow all media to come to room temperature before inoculation
2. Label all plates, tubes and slides properly with the patient's name, accession number and date.
3. Inoculate the media in the order of the least selective first to prevent carryover of inhibitory substances to another medium. Refer to the Sunquest specimen label for the order of inoculation.
4. Always inoculate the culture media first before preparing the slide when using the same pipette.

B. **Specimen processing for tubing, devices, and hardware received in a sterile container**
1. If the specimen consists of tubing, cut into 1-inch lengths using a sterile scalpel.
2. Add 1.0 ml sterile saline to the specimen cup.
3. Recap and vortex 30 seconds to rinse device, tubing sections, etc.
4. Remove the saline by using a sterile pipette.
5. Place 1-2 drops directly on each plate, into the THIO and onto a slide. Also, place a piece of tubing into the THIO tube if it fits.
6. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.
7. Save medical devices and deliver to the Histology lab for storage.
8. Streak plates semi-quantitatively for primary isolation.
   a. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
   b. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
   c. Flame the loop, turn the plate a quarter turn and pass the loop through the edge of the first quadrant approximately 4 times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant 3-4 times.
   d. Flame loop again, turn the plate another quarter of a turn, and pass the loop through the edge of the second quadrant approximately four times while streaking into the third quadrant. Continue streaking in the third quadrant without going back into the second quadrant 3-4 times.



C. **Incubation**
1. Incubate CHOC, SB, and CNA (if used) in 4-10% CO_2 at 35ºC
2. Incubate MAC (if used) and THIO in ambient air incubator at 35ºC.

D. **Gram stain examination**
1. Perform Gram stain and interpret.
2. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
3. Blot excess oil. Hold slide for one week.
4. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.

E. **Culture examination: Read plates daily for 2 days**.
1. Day 1
   a. Examine aerobic plates and THIO.

b. Plated media
   1. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase.
   2. Correlate colony types with the direct Gram stain.
   3. Use the initial Gram stain to help determine the extent of work-up required on the culture. The presence of many WBCs indicates an infectious process.
   4. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
   5. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
   6. Subculture organisms that are not well isolated to appropriate media for further work-up.
   7. Re-incubate primary plates and subcultures for an additional day.
   8. Report preliminary results.

c. THIO broth
   1. Visually inspect THIO.
   2. If growth is observed, perform gram stain on THIO.
   3. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the smear correlates with the growth on the plates. Discard after 2 days.
   4. Subculture THIO's that are turbid when plates are negative for growth and Gram stain is no organisms seen.
   5. If there appears to be additional organisms in the THIO that are not on the plates, determine if Anaerobic Culture has been ordered.
      - If Anaerobic Culture has **not** been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
      - If Anaerobic Culture has been ordered, subculture to appropriate aerobic media. Identify appropriate organisms. If the organism in THIO appears to be an anaerobe, hold THIO for 5 days.
      - After 4-5 days, confirm isolation of organism in Anaerobic Culture before finalizing culture.

2. Day 2
   a. Examine primary plates from the previous day for additional microorganisms.
   b. Read and record identification tests and susceptibilities from the previous day.
   c. Set up additional tests as needed.
   d. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section 'c' above for further instructions.
   e. Ensure THIO with growth was gram stained for 2 consecutive days.
   f. File updated report.
   g. Call MRSA results to the patient's caregiver.

3. Additional Days
   a. Complete identification and susceptibility testing procedures until all significant isolates are finished.
   b. Send updated report and finalize.
   c. If there is no growth on the plates, discard after 2 days. Culture is held open while THIO continues to incubate.
   d. Hold the THIO for 5 days. If THIO is no growth, final culture as "No Growth, 5 days".
   e. Save a representative primary plate, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.
   h. Save a representative primary plate for anaerobes in an anaerobic jar or bag, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.

Result Reporting

1. **CULTURE RESULTS**: Record culture results and culture work-ups in Sunquest MRE *Culture Entry* tab in Observations or Workups by using customized keyboards or by entering a code in the result box. Report results semi-quantitatively, i.e., 1+, 2+, 3+ or 4+.

	1st quadrant	2nd quadrant	3rd quadrant

Quantity	# colonies	# colonies	# colonies
1+	<10		
2+	>10	<5	
3+	>10	>5	<5
4+	>10	>5	>5

a. **No Growth Cultures:** Update culture status in the Observation result box (Culture Entry tab), by using the "No Growth" update key (‘). Final status: Report as "No growth 2 days".

b. **Positive cultures:**

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to follow

Workups:

Wkup # 1	Workup Components
Med: SB	GMS: STPH
Desc: BH	SC: SB
Id: SAUR	SLC: POS
	VMIC: 1
	MSID :1

c. **Gram stains**: Report Gram stain results by selecting the *Direct Exam* tab. Follow Gram Stain procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI
2. 4+ WBC'S

d. **If growth is only in the THIO**, report as:

Observations: 1. GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow (**GNR-BO-FID**)

Workups:

Wkup # 10	Workup Components
Med : THIO	SC : SB MAC
Desc : CLDY	GMS : GMNR
ID : GNR	

e. MRSA isolation requires a "Called to" if not from E.D. (disch.), or a repeat isolate. Document date and time called in computer.

Observations: 1. 3+ METHICILLIN-RESISTANT STAPH AUREUS ***MDRO***
2. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.
3. **Called to Linda S., RN L8 @ 1300 7/7/03

f. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report. The code SRPT (supplementary report) must be used in SREQ or *Culture Observations* as follows:
- Updated or new culture information: In the *Culture Entry* tab, enter SRPT on an observation line followed by new results.
- Requests for additional testing: In the *Misc. Updates* tab, enter SRPT in SREQ followed by the request.
- Re-final the culture when identifications and/or testing are complete.

g. If a culture requires a correction, the code CORR (corrected report) must be reported on an observation line in the *Direct Exam* or *Culture Entry* tab. Refer to policy MCVI 5.1 Mislabeled / Unlabeled and Correcting Patient Data for Sunquest report entry information.

References

1. Leber, Amy. Clinical Microbiology Procedures Handbook, 4th edition. Vol. 1-3. 2016. American Society for Microbiology, Washington D.C., 20036.
2. Versalovic, James, et al., *Manual of Clinical Microbiology,* 10th edition, 2011, ASM Press, American Society of Microbiology, Washington D.C. pg. 875.

Appendices

WORKLABEL MEDIA-FORM DEFINITION

BATTERY: MMC

SPEC	MEDIA
0	CHOC, SB, CNA, MAC, THIO, GMST

Training Plan/ Competency Assessment

Training Plan	Initial Competency Assessment
1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	1. Direct observation.

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Becky Carlson	09/01/1993	Initial Version
1.1	Becky Carlson	01/29/2010	PC format
1.2	Jessica Craig	05/28/2010	Updated into online format.
2	Becky Carlson	4/16/2015	Re-numbered from MC 422 for CMS load
3	Susan DeMeyere	9/8/2017	Changed reporting to keep culture open while THIO is incubating.
4	Susan DeMeyere	10/31/2018	Removed culturing for anaerobes on initial set up. Added instructions for THIO processing.
5	Susan DeMeyere	11/2/2020	Removed SCANT from reporting with growth only in THIO.
6	Susan DeMeyere	1/29/2025	Added instructions to bring medical devices to Histology after processing.