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| Miscellaneous Culture |
| **Purpose** | This procedure provides instruction for MISCELLANEOUS CULTURE for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists/Virologists who perform culture set-up and plate reading. |
| **Principle 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. |
| **Test Code** | MMC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
 | * Glass slide (GMST)
* Anaerobic Gas Pack
* Sterile disposable pipette
* Sterile container/tube
 | * Ambient air incubator
* Anaerobic jar
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
* Vortex mixer
 | Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media.* Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* Anaerobic Sheep Blood agar (ASB2)
* Anaerobic Kanamycin-Vancomycin agar (AKV)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Thioglycollate (THIO)
* Saline, Normal 1.0 mL (SLNE)
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|  |  | **Related document** |
| Sample | 1. 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
1. SDES codes/Specimen type- use free text to describe, if no code available, see examples below
* TUB – Shunt tubing
* HT-; Heart patch
* PORT-; hub (port-a-cath hub)
* BRA-; epilepsy grid (brain, epilepsy grid)
* State specific site of specimen. If the SDES CODE is unknown, do a keyword look-up at the SDES prompt by clicking on the **Result code lookup**button. Type in text and do a search by description. Highlight code and click on the **Add to list**button. Select the highlighted code to enter in SDES.

Text: ABDSearch option ○ Code ◙ DescriptionABD ABDOMENABDF ABDOMINAL FLUIDClick **Add to List*** Free text may be added to the specimen description code by clicking on Free Text. Type free text in the box and click OK. This will automatically append the free text on to the SDES code. Click on Select to save.
1. Specimen Collection and Transport
2. Refer to Lab Test Directory – Miscellaneous Culture and Gram Stain
3. Specimen assessment

1. Refer to the Specimen Rejection section of Lab Test Directory – Miscellaneous Culture and Gram Stain1. Special instructions
2. Culture within 2 hours of receipt in lab
 | [Lab Test Directory – Miscellaneous Culture and Gram Stain](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033689.asp) |
| **Special Safety Precautions** | Microbiologists/virologist are subject to occupational risks associated with specimen handling. Refer to the safety policies**:*** *Biohazard Containment*
* *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
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| **Procedure** | InoculationWarm all media before inoculation.Label all plates, tubes and slides properly with the patients name, accession number and date. 1. 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.

Always inoculate the culture media first before preparing the slide when using the same pipette.Specimen processing for tubing, devices, and hardware received in a sterile container1. Open container. Remove cover.
2. If specimen consists of tubing, cut into 1-inch lengths using a sterile scalpel.
3. Add 1.0 ml sterile saline to the specimen cup.
4. Recap and vortex 30 seconds to rinse device, tubing sections, etc.
5. Remove the saline by using a sterile pipette.

Place 1-2 drops directly on each plate, into the THIO and onto a slide. Also, place a piece of tubing or the device into the THIO tube if it will fit.1. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.
2. Streak plates semi-quantitatively for primary isolation.
3. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
4. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
5. 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.
6. 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.
	1. Incubation

1. Incubate CHOC, SB, and CNA (if used) in 4-10% CO2 at 35ºC
2. Place ASB2 and AKV (if used) in anaerobic holding chamber to be closed in an AnaeroPack™ System. Close for 2 days.
3. Place MAC (if used) and THIO in ambient air incubator at 35ºC. Hold THIO for 5 days.
	1. Gram stain examination

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
2. Blot excess oil. Hold slide for one week.
3. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
	1. Culture examination: Read plates daily for 2 days.
4. Day 1
5. Examine aerobic plates and THIO.
6. Plated media
7. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase.
8. Correlate colony types with the direct Gram stain.
9. 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.
10. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
11. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
12. Subculture organisms that are not well isolated to appropriate media for further work-up.
13. Reincubate primary plates and subcultures for an additional day.
14. Report preliminary results.
15. THIO broth
16. Visually inspect THIO.
17. If positive, smear THIO for 2 consecutive days.
18. 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.
19. If there appears to be additional organisms in the THIO that are not on the plates, subculture to appropriate media.
20. Day 2
21. Examine primary plates from the previous day for additional microorganisms.
22. Examine anaerobic plates, compare to aerobic plates, and do appropriate subcultures and identifications.
23. Read and record identification tests and susceptibilities from the previous day.
24. Set up additional tests as needed.
25. File updated report.
26. Call MRSA results to the patient’s caregiver according to [Critical and Significant Result Policy](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%20300%20%20%20%20Critical%20Results%2C%20Infection%20Prevention%20%20%26%20EIP%5CMC%20301%20%20%20Micro%20Viro%20Critical%20and%20Significant%20Result%20Notification%20R.doc).
27. Freeze isolate for future reference.
28. Additional Days
29. Complete identification and susceptibility testing procedures until all significant isolates are finished.
30. Send updated report and finalize.
31. If there is no growth on the plates, discard after 2 days. Culture is held open while THIO continues to incubate.
32. Hold the THIO for 5 days. If THIO is no growth, final culture as “No Growth, 5 days”.
33. 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.
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| **Method Performance Specifications** | *Proprionibacterium acnes* is a significant pathogen in shunt tubing cultures. Specimens will be routinely screened for rapid growing anaerobes (e.g., *Bacteroides fragilis* group, *Clostridium perfringens*, *Fusobacterium*, and anaerobic gram-positive cocci). Anaerobic organisms will be characterized or identified depending on the nature of the culture. |
| **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 semiquantitatively, i.e., 1+, 2+, 3+ or 4+.

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| Quantity | 1st quadrant# colonies | 2nd quadrant# colonies | 3rd quadrant# colonies |
| 1+ | <10 |  |  |
| 2+ | >10 | <5 |  |
| 3+ | >10 | >5 | <5 |
| 4+ | >10 | >5 | >5 |

1. 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".
2. Positive cultures:

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to followWorkups: Wkup # 1 Workup Components Med: SB GMS: STPH Desc: BH SC: SB Id: SAUR SLC: POS TUC: VMIC: 1 FOXS: 25-SS DTEST: POSIf growth is only in the THIO, report as:Observations: 1. SCANT GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow (**SCAN-GNR-BO-FID**)Workups: Wkup # 10 Workup Components Med : THIO SC : SB MAC (Add Wkld: 2) Desc : CLDY GMS : GMNR ID : GNR1. 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 7/7/03If 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.Refinal the culture when identifications and/or testing are complete.1. 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 *LABELING ERRORS/SPECIMEN MIXUPS AND CORRECTING PATIENT DATA* for Sunquest report entry information.
2. 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 |
| **References** | 1. Pezzlo, M., Section 1, Aerobic bacteriology, 3.1.1, *In* Garcia, Lynne (ed) *Clinical Microbiology Procedures Handbook*, 2010, American Society for Microbiology, Washington, D.C.
2. Versalovic, James, et al., *Manual of Clinical Microbiology,* 10th edition, 2011, ASM Press, American Society of Microbiology, Washington D.C. pg. 875.
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| **Appendices** | WORKLABEL MEDIA-FORM DEFINITIONBATTERY: MMCSPEC MEDIA0 CHOC, SB, ASB2, CNA, MAC, THIO, GMSTGASP CENT, CHOC, SB, CNA, MAC, GMSTHYP THIOINTR BPNKNGF CENT, CHOC, SB, CNA, MAC, 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.
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| **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.  |  |  |
| **Archived by:** |  | **Archived Date:** |  |