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| Ear Culture |
| **Purpose** | This procedure provides instructions for EAR CULTURE for the Microbiology laboratory. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| Principle and Clinical Significance | Acute otitis media is an infection of the middle ear and is a common pediatric disease. Uncomplicated otitis media does not require confirmation by culture; however persistent infection may require fluid collection by tympanocentesis for culture of the specific organism causing the infection. Hearing loss and deficits in learning are a few of the complications. The most common agents of otitis media are *Streptococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis,* and *Alliococcus otitis.* Otitis externa is an infection of the external auditory canal. Infections are classified as acute and chronic. Acute infections are referred to as “swimmers ear.” *Pseudomonas aeruginosa* is the most common cause of freshwater otitis, and *Vibrio alginolyticus* is a cause in oceanic otitis. Acute localized infections in the form of a pustule or furnacle are generally caused by *S. aureus* and erysipelas is caused by *S. pyogenes.* More invasive infections are caused by the progression of the bacteria into the adjacent soft tissue and bone. *P. aeruginosa* is generally the causative agent and treatment with systemic drugs is required. Chronic infections are usually caused by fungi, mycobacteria, *Nocardia*, and anaerobes. |
| **Test Code** | EARC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
 | * Glass slide (GMST)
* Anaerobic gas pack
* Sterile disposable pipette
* Sterile container/tube
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
 | * Normal Saline, 1 mL (SLNE)
* Chocolate agar (CHOC)
* Sheep Blood Agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
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| **Sample** |  |
|  |  | **Related document** |
|  | 1. Acceptable specimens
* Aspirate (tympanocentesis) for otitis media; moist swab for otitis externa. In cases of otitis media in which the eardrum has ruptured, a swab may be used to collect the exudate. Mastoid cultures are generally collected on swabs in surgery.
1. Special instructions
* Cultures for specific organisms such as *Pseudomonas, Haemophilus, Mycobacterium, or* yeast should be stated upon requisition.
 | [Lab Test Directory – Ear Culture and Gram Stain](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033051.asp) |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. *Biohazard Containment*
2. *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
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| **Procedure** | 1. Inoculation
	1. Warm all media before inoculation.
	2. Label all plates, tubes and slides properly with the patients 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 appropriate media and the order of inoculation.
	4. Always inoculate the culture media first before preparing the slide when using the same pipette.
2. Specimen processing
	1. Aspirates and exudates
		1. Place ½ to 1drop directly on each plate and onto a slide.
		2. If the specimen is received in a syringe and the volume is small, rinse syringe with a small amount of saline to remove the specimen from the syringe. Mix well.
		3. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick.
	2. Specimens received on swabs
		1. Emulsify swab in 1 ml of SLNE by vortexing well. Squeeze the swab against the side of the tube to express remaining fluid and then discard.
		2. Place1-2 drops of the suspension directly on each plate and onto a slide.
	3. Clotted specimen
		1. Put clotted specimen into a sterile tissue grinder or stomacher bag.
		2. Add 1 ml SLNE and gently homogenize to disperse clot and release bacteria.
	4. Streak plates semi-quantitatively for primary isolation.
		1. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
		2. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
		3. 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.
		4. ~AUT0029Flame 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.
3. Incubation
4. Incubate CHOC, SB and CNA in 4-10% CO2 at 35ºC.
5. Incubate the MAC in ambient air incubator at 35ºC.
6. Gram stain examination

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes according to Gram Stain procedure.
2. Blot excess oil from slide. 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.
4. Culture examination
5. Day 1
6. Examine primary plates.
7. External otitis: examine plates for 48 h for growth of enteric gram-negative rods, pseudomonads, streptococci, coryneforms, and *S. aureus.*
8. Otitis media: examine plates for 4 days for cultures of middle ear fluid. Since the specimen is collected by an invasive procedure, any organism can be considered the causative agent.
9. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
10. Correlate colony types with the direct Gram stain.
11. 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. Epithelial cells represent contamination.
12. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
13. Perform antimicrobial susceptibility testing on up to 2 PP if the organism is moderate to predominate. Perform beta-lactamase testing only on *H. influenzae.*
14. Subculture organisms that are not well isolated to appropriate media for further work-up.
15. Re-incubate primary plates and subcultures for an additional day.
16. Report preliminary results.
17. Day 2
18. Examine primary plates from the previous day for additional microorganisms.
19. Read and record identification tests and susceptibilities from the previous day.
20. Set up additional tests as needed.
21. Send updated or final report.
22. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Freeze isolate for future reference.
23. If there is no growth on the plates, continue to examine plates up to 4 days.
24. Final the report as “No Growth, 4 days”.
25. 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.
26. Additional Days
27. Complete identification and susceptibility testing procedures until all significant isolates are finished.
28. Send updated report and finalize.
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| **Method Performance Specifications** | 1. Common causes of acute otitis externa are *S. aureus, S. pyogenes,* *P. aeruginosa* (freshwater) and *V. alginolyticus* (oceanic)*.*
2. Common causes of acute otitis media are *S. pneumoniae, H. influenzae, S. pyogenes,* RSV, and influenza virus. *M. catarrhalis* *Alliococcus otitis* and *S. aureus* are encountered but are less frequent.
3. Anaerobes can cause chronic otitis media and have been implicated in chronic otitis externa.
4. *Proteus* sp. can be an important pathogen in patients with diabetes mellitus.
5. *Aspergillus* and *Candida albicans* have been implicated in chronic infections.
6. Normal ear flora includes coagulase-negative staphylococci, *Corynebacterium* sp., and viridans streptococci.
7. *Turicella otitis* is a long coryneform rod and has been implicated in otitis media. It is catalase positive, asaccharolytic and is CAMP positive.
8. *A. otitis* is slow growing and produces pinpoint colonies that are slightly yellow. They do not grow on CHOC and on Gram stain appear as gram-positive cocci in clusters and tetrads resembling staphylococci. The following test can be used for identification:
	1. Catalase negative or weak
	2. PYR positive
	3. LAP positive
	4. Vancomycin sensitive.
9. *Bordetella trematum* is an oxidase-negative, catalase-positive, fastidious gram-negative rod that has been implicated in ear infections. It is motile, frequently reduces nitrates and may or may not grow on MAC.
10. *V. alginolyticus* is an oxidase and indole positive gram-negative rod that grows on well on MAC.
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| **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+.

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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 (‘). Report as “No growth “*x*” days". Final ( / ) culture at 2 or 4 days depending on site.
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. 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'S1. Review **Culture Summary** for accuracy before filing report.
2. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Document date and time called in computer.

 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 the procedure *Labeling Errors/Specimen Mix-ups and Correcting Patient Data*
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| **References** | 1. York, M., Section 3, Aerobic bacteriology, 3.11.5, *In* L.S. Garcia (ed) *Clinical Microbiology Procedures Handbook*, 2010, American Society for Microbiology, Washington, D.C.
2. Forbes, B.A., et al., Bailey & Scott’s *Diagnostic Microbiology*, twelfth edition, 2007. Mosby, Inc., St. Louis, MO., pg. 923-926.
3. Versalovic, James, et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C., pg 59-63, 314.
4. Feigin, R.D. and J.D. Cherry, *Textbook of Pediatric Infectious Diseases*, 1998, W. B. Saunders Co., Philadelphia, PA, pg. 192-198.
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| **Appendices** | WORKLABEL MEDIA FORM DEFINITIONBATTERY: EARCSPEC MEDIAEAR SLNE, CHOC, SB, CNA, MAC, GMSTEARE SLNE, CHOC, SB, CAN, MAC, GMSTEARI SLNE, CHOC, SB, CNA, MAC, GMSTMAST SLNE, CHOC, SB, CNA, MAC, GMSTMEC SLNE, 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 | Pat Ackerman | 1973 | Initial Version |
| 1.1 | Pat Ackerman | 11/1982 |  |
| 1.2 | Pat Ackerman | 01/1992 |  |
|  | 1.3 | Pat Ackerman | 07/17/2003 |  |  |  |
| 1.4 | Pat Ackerman | 11/28/2004 |  |
| 1.5 | Pat Ackerman | 07/21/2007 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Changed TSB to SLNE. Revised label information. |
| 1.6 | Jessica Craig | 05/25/2010 | Updated into online format. |
| 2 | Becky Carlson | 4/16/2015 | Re-numbered from MC 415 for CMS load. |
| 3 | Susan DeMeyere | 10/31/2018 | Remove anaerobic culturing. |
| **Archived by:** |  | **Archived Date:** |  |