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| Eye Culture |
| **Purpose** | This procedure provides instruction for Eye Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | The eye may be involved in a variety of infections and infestations, some with or because of systemic disease. Many are vision threatening and some have implications for generalized disease. There is considerable overlap in many of these infections because many organisms affect several components of the eye. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | EYEC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Gram Stain reagents
 | * Glass slide (GMST)
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Inoculating loop
* Microscope
 | Refer to the Sunquest specimen label for media information.* Normal saline, 1 mL (SLNE)
* Chocolate agar (CHOC)
* Modified Thayer Martin agar (MTM) for newborns < 10 days old
* Sheep blood agar (SB)
* Thioglycolate (THIO)
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| **Specimen** | 1. Acceptable specimens
* Swab of conjunctiva
* scrapings of the cornea
* OD = right eye
* OS = left eye
1. Special instructions
* Specify organism suspected since special isolation procedures may be required. This procedure will not detect *Chlamydia, Mycobacterium* sp., or viruses that may cause conjunctivitis and/or keratitis.
* Because the volume of corneal scrapings and vitreous fluid aspiration is very small, direct inoculation of the agar plates and smear preparation in the clinic or at the bedside is recommended.

Refer to the Lab Test Directory for additional information: [Eye Culture and Gram Stain](https://www.childrensmn.org/References/Lab/microbioviral/eye-culture-and-gram-stain.pdf) |
| **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***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
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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.
2. Always inoculate the culture media first before preparing the slide when using the same swab.

Specimen processing1. 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. If a scant amount of ocular fluid is received in a syringe, use TSB or SLNE to wash out the syringe by drawing up a small of broth.
3. Place1-2 drops of the suspension directly on each plate, into a THIO and onto a slide.
4. Streak plates semi-quantitatively for primary isolation.
5. Sterilize the inoculating loop in the incinerator for 5 s to 10 s. Allow the loop to cool.
6. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
7. 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.
8. ~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.
9. **Incubation**
10. Incubate CHOC and SB in 3 - 5% CO2 at 35ºC.
11. Place THIO in ambient air incubator at 35ºC. Hold THIO for 5 days.
12. **Gram stain examination**

Perform Gram stain and interpret.1. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
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 for 3 days and THIO for 5 days.
7. Plated media
8. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, etc.
9. Correlate colony types with the direct Gram stain.
10. 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.
11. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
12. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
13. Subculture organisms that are not well isolated to appropriate media for further work-up.
14. Re-incubate primary plates and subcultures for an additional day. Hold MTM plate for 72 hours if on newborn ≤10 days old.
15. Report preliminary results.
16. THIO broth
17. Visually inspect THIO.
18. If growth is observed, perform gram stain on THIO.
19. 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 THIO after 2 days.
20. 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 been ordered, subculture to appropriate aerobic media. Identify appropriate organisms. If 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. If Anaerobic Culture has not been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
21. Day 2
22. Examine primary plates from the previous day for additional microorganisms.
23. Read and record identification tests and susceptibilities from the previous day.
24. Set up additional tests as needed.
25. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section‘d’ above for further instructions.
26. Ensure THIO with growth was gram stained for 2 consecutive days.
27. Send updated or final report.
28. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Freeze MRSA isolates for future reference.
29. If there is no growth on the plates, they can be tossed at 3 days. Culture is held open while THIO continues to incubate.
30. Hold negative THIO for 5 days. If no growth in THIO, final the report as “No Growth, 5 days”.
31. 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.
32. Additional Days
33. Complete identification and susceptibility testing procedures until all significant isolates are finished.

Send updated report and finalize. |
| **Method Performance Specifications** | 1. Common causes of conjunctivitis:
* *H. influenzae*
* *S. aureus*
* *S. pneumoniae*
* *S. pyogenes*
* *Neisseria gonorrhoeae*
1. Causes of infections in immune-compromised patients:
* Members of the family Enterobacteriaceae
* *Pseudomonas aeruginosa*
1. The leading cause of neonatal conjunctivitis is *C. trachomatis*. Other causes are *N. gonorrhoeae, S. aureus, Streptococcus sp., Haemophilus sp., P. aeruginosa, Moraxella sp., B. catarrhalis, N.* *meningitidis, E. coli*, and *E. cloacae*. Viruses can also cause conjunctivitis.
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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 5 days.
2. Report negative *N. gonorrhoeae* culture results on newborns ≤10 days old: Use codes **NGC1, NGC2** and **NGC3.**
3. **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 MSID : 1 **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 : GNR1. If anaerobes are isolated from broth only, do not report scant amount if plated media (ASB2) was not set up. Report as present only.
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 COCCI1. 2. 4+ WBC'S
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.
* Re-final the culture when identifications and/or testing are complete.

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*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.1%20Labeling%20Errors-Specimen%20Mix-up.docx) |
| **References** | 1. Versalovic, James., et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C.
2. Leber, Amy Section 3, Aerobic bacteriology, 3.10, *Clinical Microbiology Procedures Handbook*, 2016, 4th edition, American Society for Microbiology, Washington, D.C.
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| **Appendices** | WORKLABEL MEDIA FORM DEFINITIONBATTERY: EYECSPEC MEDIA0 SLNE, CHOC, SB, THIO, GMST, MTMCONJ SLNE, CHOC, SB, THIO, GMST, MTMEYE SLNE, CHOC, SB, THIO, GMST, MTM |
| **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 | 07/18/2003 |  |  |  |
| 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. Added hyperlink to labeling policy. |
|  | 1.6 | Jessica Craig | 05/25/2010 | Updated into online format. |
|  | 2 | Becky Carlson | 4/16/2015 | Re-numbered from MC 417 for CMS load |
|  | 3 | Susan DeMeyere | 9/7/2017 | Changed reporting to keep culture open while THIO is incubating.  |
|  | 4 | Susan DeMeyere | 10/30/2018 | 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 from THIO. Hold negative plates for 3 days.  |