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| Endotracheal Culture |
| **Purpose** | This procedure provides instruction for Endotracheal Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | Nosocomial pneumonia affects 9-21% of patients receiving mechanical ventilation for more than 72 hours. The associated mortality, reaching as much as 50%, makes nosocomial pneumonia one of the most serious complications in this patient population. Aspiration through the endotracheal tube is a noninvasive technique for obtaining respiratory secretions for quantitated culture and can be used in the assessment of suspected pneumonia in mechanically ventilated patients. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | ETC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 10% sodium desoxycholate reagent
* 3% hydrogen peroxide
* Gram Stain reagents
* Oxidase reagent
* Staphaurex™
* PBP2a
 | * Glass slide
* Inoculating loop (0.001 µL)
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Microscope
* Vortex mixer
* Vitek 2XL
* Vitek MS
 | Refer to the Sunquest specimen label for media information* Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
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| **Specimen** | 1. Acceptable specimens
* Endotracheal aspirate
1. SDES codes/Specimen type
* ETT – endotracheal aspirate
1. Refer to Lab Test Directory for Specimen Collection and Transport– [Endotracheal Aspirate Culture and Gram Stain](https://www.childrensmn.org/References/Lab/microbioviral/endotracheal-aspirate-culture-and-gram-stain.pdf)
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| **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** | 1. **Inoculation**
	1. Warm all media before inoculation.
	2. Label all plates properly with the patients name, accession number and date.
2. **Specimen processing**
3. Vortex specimen until well homogenized.
4. Using the calibrated loop method, inoculate 0.001 ml of specimen onto CHOC, SB, CNA and MAC.
5. Make a straight line down the center of plate with a loopful of specimen. Do not flame loop.
6. Streak plate by making a series of passes at 90° angles to the inoculum.
7. Rotate plate 45° and streak plate evenly over entire surface.
8. Rotate plate 45° again and repeat streaking.
9. Sterilize loop between plates in incinerator for 5 to 10 s. Cool.

a. b. c. d.~AUT00011. Place one drop of well-mixed specimen on labeled slide for Gram stain and spread evenly.
2. **Incubation**
3. Incubate CHOC, SB and CNA in 4-10% CO2 at 35ºC.
4. Place MAC in ambient air incubator at 35ºC.
5. **Gram stain examination**
6. Perform Gram stain and interpret.
7. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes according to [Gram Stain](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%202%20Staining%5CMC%202.0%20Gram%20stain.docx) and [VAP](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%202%20Staining%5CMC%202.10%20ETC-%20TRAC-%20BRC%20Gram%20Stain%20reporting%20for%20VAP.docx) procedure.
8. Blot excess oil from slide. Hold slide for one week.
9. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
10. **Culture examination**
11. Day 1
12. Examine primary plates. If growth occurs, estimate the number of colonies per ml of each organism.
13. Press the count indicator key (,), followed by the number of colonies. Multiply the number of colonies by X 1000 using the terminator key CLML (.) on the RESP keyboard.
14. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, bile solubility, etc.
15. Correlate colony types with the direct Gram stain.
16. 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.
17. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
18. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
19. Subculture organisms that are not well isolated to appropriate media for further work-up.
20. Re-incubate primary plates and subcultures for an additional day.
21. Report preliminary results.
22. Day 2
23. Examine primary plates from the previous day for additional microorganisms.
24. Read and record identification tests and susceptibilities from the previous day.
25. Set up additional tests as needed.
26. Send updated or final report.
27. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate and freeze isolate for future reference.
28. 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.
29. Additional Days
30. Complete identification and susceptibility testing procedures until all significant isolates are finished.
31. Send updated report and finalize.
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| **Interpretation** | 1. Likely to be significant:
2. Predominant potential pathogen in Gram stain and culture. Neutrophils abundant in Gram stain.
3. Potential pathogen in quantities of 104 - >106 CFU/ml.
4. Potential pathogen within neutrophils (intracellular bacteria).
5. Not likely to be significant
6. Potential pathogen <10,000 CFU/ml.
7. Neutrophils not abundant in Gram stain.
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| **Method Performance Specifications** | 1. Identify all organisms, by doing presumptive tests such as: gram stain, catalase, Staphaurex™, etc.
2. Definitively identify all potential pathogens (PP) regardless of quantity.
3. Perform AST up to 2 PP if the organisms are moderate to predominant.
* A single morphotype of a gram-negative rod.
* *S. pneumoniae, P. aeruginosa, Acinetobacter, S. aureus*
* Coagulase-negative staphylococci in neonates
* Report beta-lactamase only on *H. influenzae.*

 1. Streptococci
2. Alpha-hemolytic (AHS)
* Perform VITEK MS for identification or
* Perform direct bile solubility test on AHS and colonies that resemble *S. pneumoniae.*
* If bile solubility is questionable, perform optochin test.
* Approximately 20% of *S. pneumoniae* are resistant to bile and approximately 20% are resistant to optochin. No one test is 100% and the combination of these two tests can help prevent misidentification.
* Subculture to 2 SB with optochin disk. Incubate one in CO2 and the other in O2. *S. pseudopneumoniae* is characterized as resistant to optochin in CO2 but susceptible to optochin in O2.
1. Beta- hemolytic (BHS)
* Perform VITEK MS for identification or
* Perform strep latex typing.
* Report Streptococcus pyogenes (BSA), Streptococcus agalactiae (BSB), C or G.
* Report mucoid strains using the codes BSA – MUCO.
1. Non-hemolytic (GHS) – no work-up
* Report *Enterococcus* if predominant.
1. *Haemophilus* species
* Identify and report if *Haemophilus* is the predominant organism,
* Perform Gram stain, β lactamase, VITEK MS, VITEK NH card, or XV factor testing. Perform antimicrobial susceptibility testing (AST).
* Send all mucoid strains to MDH for typing.
1. *Moraxella (Branhamella) catarrhalis*
* If predominant on culture or if gram-negative diplococci are predominant on Gram stain, identify on VITEK MS or perform Gram stain, oxidase and Catarrhalis Test disk.
* More than 90% of *M. catarrhalis* are beta-lactamase positive.
1. *Neisseria* species
* If colony morphology resembles *N. meningitidis*, perform Gram stain, oxidase and VITEK MS or NH card. Send to MDH for typing if *N. meningitidis.*
1. Gram-negative rods
* Refer to following chart for work-up of gram-negative rods.

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| No. of Colony types | Colonies of GNR | Normal flora | Action |
| 1-2 | <10,000 | Absent to <10,000 | ID and AST |
| 1-2 | 10,000 - 40,000 | Present 50,000 - >100,000 | ID only, AST on request |
| 1-2 | 50,000 - >100,000 | Present or absent | ID and AST |
| > 2 | 10,000 – >100,000 | Present or absent | ID only, AST on request |

 Abbreviations: AST, antimicrobial susceptibility testing; ID, identification1. Fastidious gram-negative rods other than *Haemophilus* sp.
* *Francisella tularensis* are gram-negative coccobacilli that grow on CHOC. The organism may grow initially on SB but will not survive subsequent subcultures to SB. They are oxidase and urease negative and weakly catalase positive. They are beta-lactamase positive. Refer to [MCVI 3.60 Bioterrorism Protocol](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.60%20BioTerrorism%20Protocol.docx).
* *Legionella* sp. are gram-negative rods that do not grow on SB. They are motile and the colonies are about the size of *Haemophilus* with a ground glass appearance. They appear as small gram-negative bacilli that stain faintly. Send to MDH for ID.
* *Pasteurella* sp. are indole positive and oxidase positive and are associated with normal mouth flora of animals. Identify with the Vitek GN card.
* *Yersinia pestis* grows as lactose-negative colonies on MAC. They may appear as pinpoint colonies on SB at 24 hour and resemble typical enteric gram-negative rods at 48 hours. They are fat gram-negative rods with bipolar staining in gram stain (safety pin appearance). Refer to [MCVI 3.60 Bioterrorism Protocol](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.60%20BioTerrorism%20Protocol.docx).
* *Eikenella* is a small gram negative rod, (matchstick), oxidase positive and catalase negative organism that smells like bleach. ID with factor testing (requires X) or Vitek NH card.
* *Capnocytophaga* is oxidase negative and catalase negative which is CO2 loving.
* *Bordetella* sp. will grow on SB. *B. parapertussis*and*B. bronchiseptica*are catalase and urease positive. They usually are visible after 48 hours. Identify with *B. parapertussis*withBPFA reagents. *B. bronchiseptica* can be identified with the Vitek GN card.
1. *Staphylococcus aureus*
* *S.aureus* isolates require either AST or PBP2a to rule out MRSA.
* If in moderate to predominant numbers, perform PBP2a and AST.
* If in low numbers, perform PBP2a.
* Report AST, if in moderate to predominant numbers.
* Report MSSA/MRSA if in low numbers, or if AST has been reported in the last two days.
* Multiple strains of *S. aureus* may be present in one specimen. Look for subtle differences in morphology.
* Small colony variants (SCV) of *S. aureus* may be present in CF patients treated with long-term trimethoprim-sulfamethoxazole (SXT). Most SCVs are thymidine dependent causing the colonies to be smaller, flatter and grayer. SCVs appear as “fried-egg” colonies or as pinpoint colonies approximately 10 times smaller than normal *S. aureus.* Because of their unusual morphology, SCVs can be easily missed. Generally, normal growth can be restored if the isolate is grown in the presence of hemin and CO2.
1. Gram positive rods
* Perform identification if predominate.
* Examine for large spore-forming gram-positive rods. Rule out *Bacillus anthracis*. Report *Bacillus cereus.*
* If numbers of colonies are few to moderate in number with predominate UOF, report for example CORYNEFORM GRAM POSITIVE RODS most closely resembling *ACTINOMYCES* SPECIES (CGPR-ACTN) based on the colony morphology, Gram stain morphology and catalase result.
* If the organism is beta-hemolytic and catalase negative, test for *Arcanobacterium*.
* If beaded gram-positive bacilli are seen on Gram stain, do an acid fast stain to consider *Mycobacterium* or *Nocardia* species.
* Identify *Rhodococcus equi* (mucoid and urease positive) from immunocompromised patients.
1. Yeast
* Perform yeast identification with Vitek MS or Vitek YST card.
1. Molds
* Identify molds.
* Perform Lacto Phenol Cotton Blue exam.
* If conidia are consistent with *Aspergillus sp*, report SUMP-ASPE.
* All other filamentous fungi: refer to MDH for identification
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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.
	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 days.
	2. **POSITIVE CULTURES**: Report the result quantitatively. The count indicator key (,) tells the system that the result is a colony count and will be multiplied by 1000. The count terminator key (.) appends the statement COL/ML to the numeric result.
2. Type key (~) (Approximately)
3. Press the count indicator key (,) followed by 100
4. Press the terminator key (.) to append CLML
5. Type key U (CNS**)**

Observation: 1. APPROXIMATELY 100000 COL/ML COAGULASE NEGATIVE STAPHYLOCOCCIWorkups: Wkup # 1 Workup Components Med : SB GMS : STPH Desc : WH SC : SB Id : STSP SLC : NEG MSID : 1 VMIC : 1  * 1. **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* 1. Review **Culture Summary** for accuracy before filing report.
	2. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Document date and time called in computer. Freeze for future reference.

3. Approximately 10,000 METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\*4. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.5. \*\*Called to Linda S., RN L8 @ 1300 7/7/03Continued reports: If there are more isolates to report, than lines in Sunquest MRE, it will be necessary to create a continued report. In Order Entry, order ETCC (Endotracheal Tube Culture Continued Report) using same date/time. Add “SEEC” to the original accession and “RCON” to the new accession. Refer to [MCVI 5.0 Micro Computer](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.0%20Micro%20Computer%20Training.docx) for complete details.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.

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 [MC 5.1 *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., pg. 319-321.
2. Marquette, C.H., H. Georges, F. Wallet, P.Ramon, F. Saulnier, R. Neviere, D. Mathien, A. Rime, and A.B. Tonnel. 1993. Diagnostic efficiency of endotracheal aspirates with quantitative bacterial cultures in intubated patients suspected of bacterial pneumonia. Comparison with the protected specimen brush. *Am. Rev. Respir. Dis.* 148: 138-144.
3. Leber, Amy, Section 3, Aerobic bacteriology, 3.11.2, *Clinical Microbiology Procedures Handbook*, 2016, 4th edition, American Society for Microbiology, Washington, D.C.
4. Elaine R. Keith, American Society of Microbiology *Journal of Clinical Microbiology* Characteristics of *Streptococcus pseudopneumoniae* Isolated from Purulent Sputum Samples. 2006
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| **Appendices** | WORKLABEL MEDIA FORM DEFINITIONBATTERY: ETCSPEC MEDIAETT QUAN, CHOC, SB, CNA, MAC, GMSTEndotrach Diagram: |
| **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 | Pat Ackerman |  | Initial Version |
| 1.1 | Pat Ackerman |  |  |
| 1.2 | Pat Ackerman |  |  |
|  | 1.3 | Pat Ackerman | 07/21/2007 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Revised label information. |  |  |
| 1.4 | Jessica Craig | 05/25/2010 | Updated into online format. |
| 2 | Becky Carlson | 4/16/2015 | Re-numbered from MC 416 for CMS load. |
|  | 3 | Susan DeMeyere | 11/2/2020 | Updated organism reporting instructions.  |
|  | 4 | Susan DeMeyere | 2/9/2021 | Added instructions for *Streptococcus pseudopneumoniae* |
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