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| Tracheal Aspirate Culture |
| **Purpose** | This procedure provides instruction for Tracheal Aspirate Culture for the Microbiology Laboratory. |
| **Policy Statements** | This procedure applies to microbiologists who perform culture set-up and/or plate reading. |
| Principle and Clinical Significance | Tracheostomy tubes compromise the defense mechanisms that protect the lower airways. Tracheostomy tubes rapidly become colonized with gram-negative rods from the environment or with repeated aspiration. In most instances, multiple potential pathogens may be isolated regardless of the clinical status of the patient. Many patients show an inflammatory response to the tracheostomy tube itself. Thus the presence of potential pathogens in a culture may or may not indicate the etiology of pneumonia. Because of this, it is difficult to determine the significance of the presence of large numbers of an organism or to the organism’s association with the inflammatory response. Interpretation of cultures has to be carefully correlated with clinical findings. |
| **Test Code** | TRAC |
| **Materials** | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 10% sodium desoxycholate reagent
* 3% hydrogen peroxide
* Catarrhalis Test disk
* Gram stain reagents
* Oxidase reagent
* Staphaurex™
* PBP2a
* Vitek® GN, GP, YST, NH and AST cards
 | * Glass slide (GMST)
* Inoculating loop
* Sterile pipette
* Sterile swab
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Microscope
* Vortex mixer
* VITEK MS
* VITEK 2XL
 | Refer to the Sunquest specimen label for media information. * Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Saline, normal 1 mL (SLNE)
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| Sample | 1. Acceptable specimens
* Tracheal aspirate
* Tracheal tubing
1. SDES codes/Specimen type
* TRA – Tracheal aspirate
* TTUB – Tracheostomy tubing
1. Refer to Lab Test Directory) for Specimen Collection and Transport instruction [Tracheal Aspirate Culture & Gram Stain](https://www.childrensmn.org/references/lab/microbioviral/tracheal-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**:**1. *Biohazard Containment*
2. [*Safety in the Microbiology Laboratory*](https://starnet.childrenshc.org/References/labsop/mcvi/safety/mcvi-3.2-safety-in-the-microbiology-lab.pdf)
* *Biohazardous Spills*
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| **Procedure** | InoculationWarm all media before inoculation.Label all plates 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.

Specimen processing1. Tracheal secretions
* Vortex specimen until well homogenized if possible.
1. Tracheal tubing
* Cut in ½” segments and vortex in 1 ml of SLNE.
1. Using a sterile swab or pipette, inoculate the media from the purulent portion of the specimen.
2. Place one drop on each plate or roll swab across the upper quadrant of the CHOC, SB, CNA, and MAC, touching all surfaces of the swab.
3. Prepare Gram stain by spreading one drop of well-mixed specimen on labeled slide or rolling swab evenly on slide. Spread the specimen on the slide to make a thin film without rubbing it. This permits a more accurate observation without breaking up the celltypes. **Poor Gram stain results will occur if the smear is too thick.**
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.
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. 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 4-5 times.
9. Make small cuts in the primary area of the SB and CNA for hemolysis. See Figure 1 for an illustrative example.

Figure 1. Semi-quantitative plate streaking (with cuts).1. **Incubation**
2. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC
3. Place MAC in ambient air incubator at 35ºC.
4. **Gram stain examination**
5. Perform Gram stain and interpret according to the [VAP gram stain](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) criteria.
6. Quantitate 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) procedure.
7. Blot excess oil from slide. Hold slide for one week.
8. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.
9. **Culture examination**
10. Day #1:
11. Examine plates.
12. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, bile solubility, etc. Bile solubility should be performed on alpha strep only if predominant and suspicious for *S. pneumoniae*.
13. Correlate colony types with the direct Gram stain.
14. Use the initial Gram stain to help determine the extent of work-up required on the culture. The presence of many WBCs indicates an inflammatory process.
15. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
16. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
17. Subculture organisms that are not well isolated to appropriate media for further work-up.
18. Re-incubate primary plates and subcultures for an additional day.
19. Report preliminary results.
20. Day #2:
21. Examine primary plates from the previous day for additional microorganisms.
22. Read and record identification tests and susceptibilities from the previous day.
23. Set up additional tests as needed.
24. Send updated or final report.
25. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Freeze for future reference.
26. 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.
27. Additional Days:
28. Complete identification and susceptibility testing procedures until all significant isolates are finished.
29. Send updated report and finalize.
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| **Method Performance Specifications** | 1. Report the presence of “USUAL UPPER RESPIRATORY FLORA” (Sunquest code **UOF**) with at least 3 different morphologies of the following bacteria:

*Actinomyces* sp. *Haemophilus influenzae*, non-typableAlpha-hemolytic streptococci, viridans grp. *Haemophilus parainfluenzae**Capnocytophaga* sp. *Lactobacillus* sp.Coagulase-negative staphylococci *Micrococcus* sp.*Corynebacterium* sp. *Moraxella catarrhalis**Eikenella corrodens* *Neisseria* sp. (not GC or NMEN)Gamma-streptococci, viridans grp. *Stomatococcus* sp.*Streptococcus pneumoniae* Beta-hemolytic streptococcus group FYeast in low numbers (1+) Small colony β strep (colonies <0.5 mm) Gram-negative rods in low numbers (1+) 1. Perform identification tests and report the following:

*Arcanobacterium haemolyticum**S. aureus**Streptococcus pyogenes**Streptococcus agalactiae* Other Beta-hemolytic streptococci, large colony (>= 0.5 mm) groups C and G *Haemophilus influenzae*, mucoid strains (send typable strains to MDH)*Neisseria meningitides**Pseudomonas aeruginosa**Acinetobacter spp.**Stenotrophomonas spp.**Burkholderia spp.**Bacillus anthracis and cereus*Molds 1. Perform identification tests on possible pathogens that are the predominate organism. These may include the following:

*Haemophilus influenzae**M. catarrhalis**Streptococcus pneumoniae**Streptococcus pseudopneumoniae*Gram negative rods*Enterococcus spp.* 1. Streptococci
2. Alpha-hemolytic (AHS)
* Report if predominant.
* Perform VITEK MS for identification or
* Perform direct bile solubility test or
* 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.
* Report *S. pneumoniae* or *S pseudopneumoniae* if predominant and perform AST.
* 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.
1. Beta- hemolytic (BHS) colonies that are 0.5 mm or larger in size.
* Report *Streptococcus pyogenes* (BSA), *Streptococcus agalactiae (*BSB), BSC, and BSG.
* Perform VITEK MS for identification or
* Perform strep latex typing.
* Do not report small colony or BSF as they are included in UOF
* Report mucoid strains using the Sunquest codes **BSA – MUCO**.
1. Non-hemolytic (GHS) – no work-up, included in UOF
* Report *Enterococcus* if predominant.
1. *Haemophilus* species
* Report if predominant on culture.
* Perform Gram stain, β lactamase, and XV factors, Vitek MS or Vitek NH® for identification.
* Send all mucoid strains to MDH for typing.

*Moraxella (Branhamella) catarrhalis** Report if predominant on culture or if gram-negative diplococci are predominant on Gram stain
* Perform Gram stain and Oxidase. Perform Vitek MS or Catarrhalis Test disk for identification.
* Use the Sunquest computer code MCAT.
1. *Neisseria* species
* Report if colony morphology resembles *N. meningitidis*.
* Perform Gram stain and Oxidase. Perform Vitek MS or Vitek NH® for identification.
* Send to MDH for typing if *N. meningitidis.*
1. Gram-negative rods
* Identify and perform AST with single morphotype of a gram-negative rod if present in significant amounts and predominant, especially *Klebsiella pneumoniae*.
* Identify and perform AST if present in significant amounts, even if not predominant because the following organisms are typically resistant to many antimicrobials and associated with nosocomial infections.
1. *Pseudomonas aeruginosa*
2. *Stenotrophomonas maltophilia*
3. *Acinetobacter sp.*
4. *Burkholderia sp.*
5. *Bordetella bronchiseptica*
* 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 | 1+ to 2+ | Absent to 1+ or if gnr predominant on GMSTAbsent | ID and AST |
| 1-2 | 1+ to 2+ | 3+ to 4+ | ID only, AST on request |
| 1-2 | 3+ to 4+ | Present or absent | ID and AST |
| > 2 | 1+ to 4+ | Present or absent | ID and AST on request |

Abbreviations: AST, antimicrobial susceptibility testing; ID, identification* Members of the *Enterobacteriaceae* are relatively uncommon causes of pneumonia but are more frequently seen in the hospitalized or debilitated patient. The isolation of these organisms may not be abnormal but represent colonization. Consult the physician before extensive identification procedures and susceptibility testing is performed on multiple organisms.
* ***Pasteurella spp:*** Perform β-lactamase testing for isolates recovered from respiratory and normally sterile sites. β-lactamase positive isolates are resistant to ampicillin, amoxicillin, and penicillin.
* Routine susceptibility testing is usually not recommended from bite wounds. Testing from normally sterile sites and respiratory specimens may be warranted. Send isolates to the U of M for susceptibility testing.
1. *Staphylococcus aureus*
* Perform PBP2a to determine MRSA.
* Perform AST on all new MRSA.
* Perform AST on MSSA if in moderate to predominant numbers.
* Small colony variant strains may be present due to long-term Trimethoprim-sulfamethoxazole (SXT) treatment. They are small, flat, gray colonies, generally MRSA.
1. Gram positive rods
* If the organism is beta-hemolytic and catalase negative, test for *Arcanobacterium*.
* If a beaded gram-positive rod is seen on Gram stain, consider *Mycobacterium* or *Nocardia* species.
* If predominant, identify *Corynebacterium* to species level.
* Identify and report *Bacillus anthracis* and *Bacillus**cereus.*
* Consider *Rhodococcus equi* in immunocompromised patients.
1. Yeast
* If there is 1+ yeast and moderate or predominant UOF, report as “USUAL UPPER RESPIRATORY FLORA”
* If there is 2+ yeast, perform yeast identification on Vitek MS or Vitek YID card.
* If yeast is in low to moderate numbers with absent to few UOF or if seen on Gram stain, perform yeast identification on Vitek MS or Vitek YID card.
* If yeast is predominating, perform yeast identification on Vitek MS or Vitek YID card.
1. Molds: Identify all molds
* Perform Lacto Phenol Cotton Blue exam. If conidia are consistent with *Aspergillus sp*, report presumptive *Aspergillus* sp, SUMP-ASPE.
* All other filamentous fungi: refer to MDH for complete ID.
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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. Culture with no predominate organism or no probable pathogens, report as follows:

Observations: 1. 4+ USUAL UPPER RESPIRATORY FLORA Sunquest code: **UOF**2. Susceptibilities not performed. Please contact Microbiology Sunquest code: **SNP** if further testing is required (MPLS 813-5866). Workups: Wkup# 1 Workup Components Med : SB  Desc: AHS-GHS-NEIS-HAEM-SMUC GMS : STR-HAE-NEIS  Id: UOF1. Culture with probable pathogens, report as follows:

Observations: 1. 4+ USUAL UPPER RESPIRATORY FLORA2. 3+ PSEUDOMONAS AERUGINOSA (MUCOID)3. 2+ PSEUDOMONAS AERUGINOSA (MATTE)4. 2+ STAPHYLOCOCCUS AUREUS Further identification to follow5. 1+ PRESUMPTIVE ASPERGILLUS SPECIES6. Susceptibilities to follow. Workups: Wkup# 1 Workup Components Med : SB Gram: GPC  Desc: AHS-GHS-NEIS-SMUC Id: UOF1. 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\*\*\* Sunquest code: **MRSA** 2. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism Sunquest code: **DRO** requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.3. \*\*Called to Linda S., RN L8 @ 1300 7/7/031. Gram stains: Report Gram stain results by selecting the *Direct Exam* tab. Follow Gram stain/ VAP procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI1. 4+ WBC'S
2. Review **Culture Summary** for accuracy before filing report.

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.
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 [MC 5.1 LABELINGERRORS/SPECIMEN MIXUP*.*](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. Leber, Amy, Section 3, Aerobic bacteriology, 3.11.2, 3.11.3, *Clinical Microbiology Procedures Handbook*, 2016, 4th edition, American Society for Microbiology, Washington, D.C.
2. Pezzlo, M., Section 2. Aerobic bacteriology, 2.6, pg. 73 - 80. *In* HD Isenberg (Ed) *Essential Procedures for Clinical Microbiology.* 1998, American Society for Microbiology, Washington, D.C.
3. Versalovic, James, et al, *Manual of Clinical Microbiology*, 2011, ASM press, American Society for Microbiology, Washington, D.C., pg. 63, 316 – 317.
4. Bartlett, J.G., N.S. Brewer, K. J. Ryan, Co.Ed. J.A. Washington, *Cumitech 7*, Laboratory diagnosis of lower respiratory tract infections, 1978, ASM Press, Washington, D.C.
5. 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 Definition: BATTERY : TRAC SPEC MEDIA 0 CHOC, SB, CNA, MAC, CUT3, GMST TTUB SLNE, VRTX, CHOC, SB, CNA, MAC, CUT3, GMST**Trach tube****ET tube** |
| **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 | 11/16/1978 |  |
| 1.2 | Pat Ackerman | 12/27/1995 |  |
|  | 1.3 | Pat Ackerman | 07/31/2003 |  |  |  |
| 1.4 | Pat Ackerman | 01/11/2008 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Added Hyper-link to Labeling policy, Gram stain procedure, Updated procedural notes: BHS, gnrs and gprs. |
| 1.5 | Becky Carlson | 07/14/2013 | Removed Strep FA confirmation. Reagent no longer available. |
| 1.6 | Tina Gronquist | 06/16/2014 | Updated into online format. |
| 2 | Becky Carlson | 4/18/2015 | Re-numbered from MC 431 for CMS load. |
| 3 | Eileen BrinkmanSusan DeMeyere | 2/5/2018 | Updated bile solubility testing on alpha streps. |
| 4 | Susan DeMeyere | 11/2/2020 | Updated organisms reporting instructions.  |
| 5 | Susan DeMeyere | 2/9/2021 | Added instructions for *Streptococcus pseudopneumoniae* |
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| **Archived by:** |  | **Archived Date:** |  |