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| Bronchoscopy Culture |
| **Purpose** | This procedure provides instruction for Bronchoscopy Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | Specimens from the lower respiratory tract are submitted to determine the cause of airway disease such as bronchitis, pneumonia, lung abscesses and empyema. Lower respiratory tract infections are a major cause of morbidity and mortality. It is difficult to diagnose these infections because specimens are often contaminated during collection by upper respiratory secretions. The upper respiratory tract may be colonized with potential pathogens that are not involved in the lower tract .The specimen should be examined microscopically to assess the quality of the specimen. A properly collected specimen will contain a minimum of squamous epithelial cells and significant numbers of PMNs. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | BRC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 10% sodium desoxycholate reagent
* 3% hydrogen peroxide
* VITEKcards
* Catarrhalis Test disk
* Gram Stain reagents
* Oxidase reagent
* PBP2a
* StaphaurexTM
 | * Glass slide (GMST)
* Pipette tips
* Sterile disposable pipette
* Inoculating loop (0.001 mL)
* Inoculating loop (10 µL) or 10 µL pipette with sterile tips
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Microscope
* Vortex Mixer
* Vitek 2XL
* Vitek MS
 | Refer to the Sunquest specimen label for media information. The specimen type determines the appropriate media.* Chocolate agar (CHOC)
* Sheep blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)

For CF patients, add:* Pseudomonas cepacia medium (PCM)
* Sabouraud with chloral & gent (SABC)
* Chrome Staph aureus agar (CSA)
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| **Specimen** | 1. Acceptable specimens
* Bronchoalveolar lavage (BAL)
* Bronchial aspirate/washing
* Bronchoscopy brushing
1. SDES codes/Specimen type:
* BR – bronch
* BRCF – bronch, cystic fibrosis
* BAL – Bronchoalveolar lavage
* BASP – Bronchial aspirate
* BRB – Bronchial brushing
* BRW – Bronchial washing
* LLL – Left lower lobe
* LMA – Left mainstem
* LUL – Left upper lobe
* RLL – Right lower lobe
* RMA – Right mainstem
* RML – Right middle lobe
* RUL – Right upper lobe

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* For additional information: Refer to [Bronchoscopy Culture & Gram Stain](https://www.childrensmn.org/References/Lab/microbioviral/bronchoscopy-culture-and-gram-stain.pdf) (Lab Test Directory) for collection and transport instruction.
1. Special instructions:
* **STAT processing**: Contact pathology at 612-813-6711 for STAT processing. Page pathologist on call if after hours.
* Specimens should be processed within 1 hour of collection.
* **Order BAAH** (Bronchoscopy/Airway Aspiration Histology) on ALL bronchoscopy specimens that have Histology orders for special stains.
* **Order EBRON** (Extra Bronchoscopy specimen, no histology) on ALL bronchoscopy specimens without Histology orders for special stains.
* If delays are unavoidable, the specimen should be refrigerated at 2-8°C up to 2 hours.
* Identify all CF patients in SDES as “Bronch, Cystic Fibrosis.” (Sunquest code: BRCF). Append specific site, i.e., BRCF-LLL.
* If a cell count is ordered, measure specimen volume and record on the bronchoscopy requisition.
* Hematology must receive the specimen within one hour of collection for cell count and/or Histology cytospin prep.
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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, tubes and slides properly with the patients name, accession number and date.
2. **Specimen processing**
3. Bronchial alveolar lavage (BAL) or bronchial aspirate/wash
* Vortex specimen until well homogenized.
* Using the calibrated loop method, inoculate 0.001 ml of specimen onto CHOC, SB, CNA, MAC and if CF requested, PCM, SABC, and CSA. Streak in order of least selective agar to most inhibitory as listed on the Sunquest media label.
1. Make a straight line down the center of plate with a loopful of specimen. Do not flame loop.
2. Streak plate by making a series of passes at 90° angles to the inoculum.
3. Rotate plate 45° and streak plate evenly over entire surface.
4. Rotate plate 45° again and repeat streaking.
5. Sterilize loop between plates in incinerator for 5 to 10 seconds. Allow to cool.

~AUT0001 1. 2. 3. 4. Figure 1. * Place one drop of well-mixed specimen onto a labeled slide. Allow to heat fix.
1. Bronchial brush
2. Place bronchial brush into 1 ml of sterile saline after collection.
3. Vortex specimen.
4. Prepare smear for Gram stain by cytocentrifugation or placing 1 drop of specimen on glass slide.
5. Inoculate 0.01 ml of specimen onto CHOC, SB, CNA, MAC, and if CF requested, CSA, SABC, and PCM by using a 10µl loop or pipette with sterile pipette tips.
6. Streak specimen evenly over entire surface of plate (See Figure 1).
7. **Incubation**
8. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC.
9. Place MAC and, if CF patient, CSA, SABC, and PCM in ambient air incubator at 35ºC.
10. **Gram stain examination**

Perform Gram stain and interpret.1. Quantitate neutrophils (NEUTR), squamous epithelial cells (SEC), histiocytes, bacterial and fungal morphotypes according to the [Gram Stain](https://starnet.childrenshc.org/References/labsop/micro/stain/mc-2.0-gram-stain.pdf) 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) procedures
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 plates. If growth occurs, estimate the number of colonies per mL of each organism.
7. BAL and bronchial aspirates/washes
* Estimate the number of colonies of each organism per mL. 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.
1. Bronchial brush
* Estimate the number of colonies of each organism per mL by multiplying X 100.
1. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, bile solubility, etc.
2. Correlate colony types with the direct Gram stain.
3. 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.
4. Set up definitive biochemical or identification procedures on significant organisms if well isolated. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
5. Subculture organisms that are not well isolated to appropriate media for further work-up.
6. Re-incubate primary plates and subcultures for an additional day.
7. Report preliminary results.
8. Day #2
9. Examine primary plates from the previous day for additional microorganisms.
10. Read and record identification tests and susceptibilities from the previous day.
11. Set up additional tests as needed.
12. Hold CF culture plates for a minimum of three days. Hold PCM for 5 days. *B. cepacia* will appear pink on the PCM.
13. Confirm *B. cepacia* identification on commercial systems with conventional biochemical tests. Send *B. cepacia* to the University of Michigan reference laboratory and freeze isolate for future reference. Alert physician.
14. Call MRSA results to patient’s caregiver, if not E.D. (disch.) or a repeat isolate. Freeze isolate for future reference.
15. Send updated or final report.
16. 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.
17. Additional Days
18. Complete identification and susceptibility testing procedures until all significant isolates are finished.
19. Send updated report and finalize.
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| **Method Performance Specifications** | 1. Identify all organisms, by doing presumptive tests such as: gram stain, catalase, Staphaurex™, etc.
* Definitively identify all potential pathogens (PP) regardless of quantity.
* Perform AST on 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* is 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*
* Identify and report 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.
2. **NO GROWTH CULTURES**: Update culture status in the Observation result box (*Culture Entry* tab), by using the “No Growth” update key (‘).
3. **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.
4. Type key (~) (Approximately)
5. Press the count indicator key (,) followed by 100
6. Press the terminator key (.) to append CLML
7. Type key R (SPNE**).**

Observation: 1. APPROXIMATELY 100000 COL/ML STREPTOCOCCUS PNEUMONIAEWorkups: Wkup# 1 Workup Components Med : SB BS : POS Desc : AH SC : SB Id : AHS GMS : STR MICS : DONE1. Report small colony variants (SCV) of *S. aureus* by appending the Sunquest code **SCV**as follows:

Approximately 10,000 METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\* (small colony variant) (APPR-10,000-CLML-MRSA-SCV)1. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Document date and time called in computer. See [MCVI 4.0 Critical Results](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%204%20Result%20Notification%5CMCVI%204.0%20Critical%20Results.docx) procedure.

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 INFECTION CONTROL @ 1300 7/7/031. **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. 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. Leber, Amy, Section 3, Aerobic bacteriology, 3.11, *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.
4. *LRN Level A Bioterrorism Laboratory Protocols,* Minnesota Laboratory System, Sections 4, 7 and 9.
5. Barbara C. Kahl, G. Belling, R. Reichelt, M. Herrmann, R.A. Proctor, and G. Peters **“Thymidine-Dependent Small-Colony Variants of *Staphylococcus aureus* Exhibit Gross Morphological and Ultrastructural Changes Consistent with Impaired Cell Separation”** J. Clin. Microbiol. 2003 41: 410-413.
6. 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: BRCSPEC MEDIA1. QUAN, CHOC, SB, CNA, MAC, GMST

BRB QUAN1, CHOC, SB, CNA, MAC, GMSTBRCF QUAN, CHOC, SB, CNA, MAC, PCM, SABC, CSA, 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 | 11/1982 | Initial Version |
| 1.1 | Pat Ackerman | 01/1992 |  |
| 1.2 | Pat Ackerman | 07/08/2003 |  |
|  | 1.3 | Pat Ackerman | 09/03/2004 |  |  |  |
| 1.4 | Pat Ackerman | 07/04/2007 | Updated Sunquest 6.2 reporting information. Modified SRPT and CORR statements. |
| 1.5 | Jessica Craig | 05/20/2010 | Updated into online format. |
|  | 2 | Becky Carlson | 4/15/2015 | Re-numbered from MC 409 |
|  | 3 | Becky Carlson | 10/5/2015 | Added order code BAAH to the Policy Statement and Special Instructions |
|  | 4 | Becky Carlson | 4/20/2016 | Added order code EBRON to the Policy Statement and Special Instructions |
|  | 5 | Susan DeMeyere | 11/2/2020 | Updated organisms reporting instructions |
|  | 6 | Susan DeMeyere | 2/9/2021 | Added instructions for *Streptococcus pseudopneumoniae* |
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