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| Throat Culture  |
| **Purpose** | This procedure provides instruction for Throat Culture for the Microbiology Laboratory. |
| **Principal and Clinical Significance** | The majority of cases of pharyngitis are of viral etiology. At least 9 different viruses have been implicated, some of them producing symptoms indistinguishable from those of streptococcal infection such as severe sore throat with ulceration, exudate, and fever. Most cases of bacterial pharyngitis (up to 98%) are caused by beta-hemolytic streptococci, primarily those in group A. There is some evidence that other beta-hemolytic streptococci, especially groups C and G and *Arcanobacterium haemolyticum* (*Corynebacterium*) may occasionally cause pharyngitis. These organisms are thought to be self-limiting and so far have not been associated with serious sequelae as seen with group A.A throat swab may also be helpful in determining the cause of epiglottitis, which can be life threatening causing airway obstruction. Epiglottitis is usually caused by Haemophilus *influenzae* type b but is occasionally caused by *S. pneumoniae* and *S. pyogenes.*Young cystic fibrosis (CF) patients usually do not produce sputum. In these patients, deep throat swabs are cultured for potential respiratory pathogens. Placing a swab in the pharynx induces coughing, resulting in lung secretions being brought up into the pharynx.Refer to the [Corynebacterium diphtheriae Culture](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMC%201%20Culture%20Procedures%5CMC%201.10%20Corynebacterium%20diphtheriae%20Culture.docx) procedure for *C. diphtheriae* information.  |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | TC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 10% sodium desoxycholate reagent
* 3% hydrogen peroxide
* Vitek® GP, GN, NH, YST and AST cards
* Catarrhalis Test disk
* Gram Stain reagents
* Strep grouping kit
* Oxidase reagent
* Staphaurex™ kit
* PBP2a
 | * Glass slides
* Inoculating loop
 | * Ambient air incubator
* Anaerobic jar
* CO2 incubator
* Incinerator
* Microscope
* VITEK MS
* VITEK 2XL
 | Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media, i.e., CF patients require a PCM, SABC and CSA.* Anaerobic Sheep Blood agar,

--1 day -- (ASB1)* Chocolate agar (CHOC)
* CNA agar (CNA)
* MacConkey agar (MAC)
* Selective Strep agar (SSBA)
* For CF and PCD patients only:
* Pseudomonas cepacia agar (PCM)
* Sabouraud with CC (SABC)
* Chrome Staph aureus (CSA)
* Serum tellurite agar (TELL) for Corynebacterium diphtheriae Culture
 |
| **Specimen** | 1. Acceptable specimens:
* Throat swab
1. SDES codes/Specimen type
* EPI – epiglottis
* SUBG – subglottis
* THCF – throat, CF
* THR – throat
* THPCD-throat, primary ciliary dyskinesia
* THRD – throat for *C. diphtheriae*
* See [*Corynebacterium diphtheriae* Culture Procedure MC 1.10](https://starnet.childrenshc.org/References/labsop/micro/cultpro/mc-1.10-corynebacterium-diphtheriae-culture.pdf)
1. Refer to the Lab Test Directory for Specimen Collection and Transport information. [Throat Culture, Routine](http://www.childrensmn.org/Manuals/Lab/MicroBioViral/033688.asp) .
 |
| **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)
 |
| **Procedure** | InoculationWarm all media before inoculation.Label all plates and slide properly with the patients name, accession number and date. 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. Roll swab across the upper quadrant of the CHOC, SB, CNA, MAC, SSBA, (CSA, SABC, PCM) touching all surfaces of the swab.
2. Streak plates semi quantitatively for primary isolation.
3. Sterilize the inoculating loop in the incinerator for 5 to10 seconds. Allow the loop to cool.
4. 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.
5. 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 3-4 times.
6. Make small cuts in the primary area of the SB for hemolysis. See Figure 1 for an illustrative example.

Figure 1. Semi-quantitative plate streaking (with cuts).1. **Incubation**
2. Incubate CHOC, CNA and SSBA in 4-10% CO2 at 35ºC
3. Place ASB1 in anaerobic holding chamber to be closed in an AnaeroPack™ or Ana bag system for 1 day.
4. Place MAC, (CSA, SABC, and PCM) in ambient air incubator at 35ºC.
5. **Culture examination**
6. Day #1:
7. Examine plates.
8. Gram stain each colony type and perform initial identification procedures as needed, i.e., catalase, oxidase, bile solubility, etc. Bile solubility only needs to be performed if the alpha strep is predominant and suspicious for *S. pneumoniae*.
9. Set up definitive biochemical or identification procedures on significant organisms if well isolated.
10. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
11. Subculture organisms that are not well isolated to appropriate media for further work-up.
12. Re-incubate primary plates and subcultures for an additional day.
13. Report preliminary results.
14. Day #2:
15. Examine primary plates from the previous day for additional microorganisms.
16. Read and record identification tests and susceptibilities from the previous day.
17. Set up additional tests as needed.
18. Hold CF culture plates for a minimum of three days. Hold PCM for 5 days.
19. Confirm *B. cepacia* identification on commercial systems with conventional biochemical tests. Send *B. cepacia* to the University of Michigan reference laboratory (see appendix) and freeze isolate for future reference. Alert physician.
20. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Freeze isolates for future reference.
21. Send updated or final report.
22. 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.
23. Additional Days:
24. Complete identification procedures until all significant isolates are finished.
25. Send updated report and finalize.
 |
| **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* Yeast in low numbers (1+) (non-CF patients)Gram-negative rods in low numbers (1+)(non-CF patients)Beta-hemolytic streptococcus group F Small colony β strep (colonies <0.5 mm) 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. See CF Notes for further information.
* If bile solubility is questionable, perform optochin test.
1. Beta- hemolytic (BHS) colonies that are 0.5 mm or larger in size.
* 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 Sunquest codes **BSA – MUCO**.
1. Non-hemolytic (GHS) – no work-up
* Report *Enterococcus* if predominant.
1. *Streptococcus pyogenes* (group A beta-hemolytic strep) is generally susceptible to penicillin and its derivatives; therefore, susceptibility need not be routinely performed. The principal reason for considering an alternative drug for individual patients is allergy to penicillin. Erythromycin, a Cephalosporin, or Clindamycin might be substituted in these cases. Patients allergic to penicillins may also be allergic to cephalosporins.
* Add Sunquest code **BHSS** to report: Beta hemolytic streptococci are susceptible to penicillin, cephalosporins and vancomycin. Some strains may be resistant to erythromycin.
* Call Children’s Respiratory Clinic with *Streptococcus pyogenes* (group A β strep) isolates.
1. *Haemophilus* species
* Report if *Haemophilus* is the predominant organism.
* Perform Gram stain, β lactamase, and VITEK MS or Vitek NH®.
* 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, oxidase and Catarrhalis Test disk or identify on VITEK MS.
* Report with Sunquest code MCAT.
1. *Neisseria* species
* Report if colony morphology resembles *N. meningitidis*,
* Perform Gram stain, oxidase and VITEK MS or Vitek NH®.
* 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

|  |  |  |  |
| --- | --- | --- | --- |
| 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 AST or PBP2a on all *Staph aureus* and report MRSA or MSSA as indicated.
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.
* Identify and report *Bacillus anthracis* and *Bacillus**cereus.*
1. Yeast
* If not predominant, report as “YEAST, No further identification.” Perform identification upon request.
* If yeast is predominant, 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 ID.

1. Traditional pathogens for cystic fibrosis (CF) patients are as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| Organism | Usual oral flora | Identify | Processing level |
| *S. aureus* a | NEVER | Any amount | Perform PBP2a and/or AST  |
| *Ps. aeruginosa* (matte and mucoid colony types) | NEVER | Any amount | Perform ID and AST **(AZT KB)** |
| *Alcaligenes xylosoxidans**Acinetobacter spp.* | NEVER | Any amount | Perform ID and AST  |
| *Burkholderia cepacia* complex c**Confirm ID with Vitek** | NEVER | Any amount | Perform ID and AST  |
| *S. maltophilia* | NEVER | Any amount | Perform ID and AST-all 3 drugsMSCN  |
| *Burkholderia gladioli,* *B. bronchiseptica, Pandoraea sp.* and *Ralstonia sp. e* | NA(Considered emerging pathogens) | Any amount | Perform ID and AST (MSCN) |
| *Enterobacteriaceae sp.* | NEVER | Any amount | Perform ID only; AST upon request |
| *H.* *influenzae* | Not predominant | Predominant | Perform ID and β-lactamase; Send to MDH for serotype if colony is mucoid or wet |
| *S. pneumoniae**S. pseudopneumoniae b* | Not predominant | Predominant | Perform AST  |
| Molds, *Aspergillus* sp. | NEVER | Any amount | Perform presumptive ID only; send to MDH if complete ID requested. AST upon request |
| Rapid growing mycobacteria | NEVER | Any amount | Identify to species level; send to MDH for ID |

**CF Notes:**1. *S. aureus* (CF):
* Multiple strains may be present in one specimen; look for subtle differences.
* Small colony variant strains may be present due to long-term trimethoprim-sulfamethoxazole (SXT) treatment. They are small, flat, gray colonies, generally MRSA. Perform AST with KB an MH with SB in CO2.
1. *S. pseudopneumoniae*
* Pathogen similar to S. pneumoniae
* Has been recognized as an opportunistic pathogen in patients with CF.
* Bile insoluble
* Resistant or intermediate to optochin in CO2.
* Susceptible to optochin in O2.
1. *Burkholderia cepacia* complex*:*
* *B. cepacia* will appear pink on PCM.
* Perform Vitek MS for identification.
* Confirm *B. cepacia* identification with Vitek.
* *S*end isolates to the *B. cepacia* Research Laboratory and Repository. See appendix for details.
* Freeze isolate for future reference. Alert physician.
1. Yeast: Report as “YEAST, No further identification.” Perform identification upon request.
2. *Burkholderia cepacia* complex, Pandoraea sp. and Ralstonia sp.: *S*end isolates to the *B. cepacia* Research Laboratory and Repository. See appendix for details.
 |
| **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+.

|  |  |  |  |
| --- | --- | --- | --- |
| 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 organisms or no potential pathogens (PP), report as follows:

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

Observations: 1. 4+ USUAL UPPER RESPIRATORY FLORA 2. 3+ PSEUDOMONAS AERUGINOSA (MUCOID) 3. 2+ PSEUDOMONAS AERUGINOSA (MATTE) 4. 2+ STAPHYLOCOCCUS AUREUS Further identification to follow 5. 1+ ASPERGILLUS SPECIES 6. Susceptibilities to follow.Workups: Wkup# 1 Workup Components Med: SB GMS: STR-HAE-NEIS 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: 3. 3+ METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\* Sunquest code: **MRSA** 4. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control. Sunquest code: **DRO** 5. \*\*Called to Linda S., RN L8 @ 1300 7/7/031. Report strains of *P. aeruginosa* as matte or mucoid using the Sunquest codes **PMUC** or **PMAT**
2. Report mucoid strains of *S. pyogenes* using the Sunquest code **BSA – MUCO.**
3. Review **Culture Summary** for accuracy before filing report.
4. 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 MIXUPS](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)for Sunquest report entry information.
5. 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.
 |
| **References** | 1. Leber, Amy, Aerobic bacteriology, 3.11.2, 3.11.3,  *Clinical Microbiology Procedures Handbook*, 2016 4th edition, American Society for Microbiology, Washington, D.C.
2. Gilligan, P.H., et.al, 2006, Cumitech 43, *Cystic Fibrosis Microbiology*, ASM Press, 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. Whittier, Susan, Update on the microbiology of cystic fibrosis: traditional and emerging pathogens. *Clinical Microbiology Newsletter*, Vol. 23, No. 9, pg. 67-71.
5. Hoppe, J.E., U. Theurer-Mainka, M. Stern, Comparison of three methods for culturing throat swabs from cystic fibrosis patients, *Journal of Clinical Microbiology,* Vol. 33, No. 7, 1995, pg 1896-1898.
6. Elaine R. Keith, American Society of Microbiology *Journal of Clinical Microbiology* Characteristics of *Streptococcus pseudopneumoniae* Isolated from Purulent Sputum Samples. 2006
7. Chloe Dupont, *Journal of Cystic Fibrosis*, *Streptococcus pseudopneumoniae*, an opportunistic pathogen in patients with cystic fibrosis. 11/20/2019
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| **Appendices** | WORKLABEL MEDIA FORM DEFINITIONBATTERY: TCSPEC MEDIA0 CHOC, ASB1, MAC, SSBA, CUT3EPI CHOC, SB, CNA, MAC, CUT3SUBG CHOC, SB, CNA, MAC, CUT3THCF CHOC, ASB1, CNA, MAC, PCM, SABC, CSA, CUT3THPCD CHOC, ASB1, CNA, MAC, PCM, SABC, CSA, CUT3THR CHOC, ASB1, MAC, SSBA, CUT3THRD SB, TELL*B. cepacia* Research Laboratory and Repository—see “white binder” on shelf for protocol and formsDr. LiPuma’s Lab -- CFF *Burkholderia cepacia* Research Laboratory and Repositoryjlipuma@umich.eduThe University of Michigan Health System1150 W. Medical Center Drive Ann Arbor, MI 48109-5646 |
| **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 | 1978 | Initial Version |
| 1.1 | Pat Ackerman | 04/1983 |  |
| 1.2 | Pat Ackerman | 04/1988 |  |
|  | 1.3 | Pat Ackerman | 07/29/2003 |  |  |  |
| 1.4 | Pat Ackerman | 12/10/2004 |  |
| 1.5 | Pat Ackerman | 08/22/2007 | Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Updated CF traditional organisms into table format. Limit yeast identification to physician request only. Added code information BHSS. |
|  | 1.6 | Jessica Craig | 06/16/2010 | Updated into online format. |
|  | 1.7  | Becky Carlson | 3/19/2014 | Updated CF table; removed mucoid GAS referral to Dr Kaplan |
|  | 1.8 | Becky Carlson | 4/18/2015 | Re-numbered from MC 429. Added SSBA to list of set up medias |
|  | 2 | Eileen BrinkmanSusan DeMeyere | **2/5//2018** | Updated bile solubility testing on alpha streps. |
|  | 3 | Susan DeMeyere | 11/2/2020 | Updated organisms reporting instructions. |
|  | 4 | Susan DeMeyere | 2/9/2021 | Added instructions for *Streptococcus pseudopneumoniae* |