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| Sputum Culture |
| **Purpose** | This procedure provides instruction for Sputum Culture for the Microbiology laboratory. |
| **Principal and Clinical Significance** | Lower respiratory tract infections are a major cause of morbidity and mortality.The examination of expectorated sputum has been the primary means of determining the cause of bacterial pneumonia. However, lower respiratory tract specimens will be contaminated with upper respiratory tract secretions, especially saliva, unless they are collected using an invasive technique. For this reason, sputum is among the least clinically relevant specimens received for culture. Sputum specimens are 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.Cystic fibrosis is a major cause of chronic pulmonary disease in children. Patients with CF have a lifetime of bacterial bronchitis, despite aggressive multiple antibiotic therapy. Typically, these children present with recurrent lower respiratory infections and very characteristic sputum bacteriology. |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. |
| **Test Code** | SPUC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * 10% sodium desoxycholate reagent
* 3% hydrogen peroxide
* Vitek® GP, GN, NH, AST cards as needed
* Catarrhalis Test disk
* Gram stain reagents
* Oxidase reagent
* Staphaurex™
* PBP2a
 | * Glass slide (GMST)
* Sterile swab
* Inoculating loop
 | * Ambient air incubator
* CO2 incubator
* Incinerator
* Microscope
* Vortex mixer
* Vitek 2XL
* Vitek MS
 | Refer to the Sunquest specimen label for media information. Appropriate media is determined by the specimen site, i.e., CF patients require a SABC, CSA and PCM.* Chocolate agar (CHOC)
* Sheep Blood agar (SB)
* CNA agar (CNA)
* MacConkey agar (MAC)
* For CF and PCD patients only:
* Pseudomonas cepacia agar (PCM)
* Sabouraud with CC (SABC)
* Chrome Staph aureus (CSA)
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| **Specimen** | 1. Acceptable specimens
* Sputum, deep cough or aspirate
1. SDES codes/Specimen type
* SPU – sputum
* SPCF – sputum, cystic fibrosis
* SPPCD-sputum, primary ciliary dyskinesia
1. Refer to the Lab Test Directory for Specimen Collection and Transport- [Sputum culture](https://www.childrensmn.org/References/Lab/microbioviral/sputum-culture-and-gram-stain.pdf)
2. Do not reject sputum specimens from CF patients based on the Gram stain results. 5-10% of these patients may have difficulty producing an expectorated specimen.
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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)
 |
| **Procedure** | Gram stain examination1. Prepare a Gram smear from the purulent portion of the specimen. Roll the swab over the 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 cell types. Poor Gram stain results will occur if the smear is **too thick**.
2. Stain smear and examine the slide under low power (10X objective) to access specimen quality and quantitate WBCs, epithelial cells, and histiocytes cells. Refer 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.
	1. Examine a minimum of 20-40 fields concentrating on areas with WBCs.
	2. Average the number of cells in representative fields that contain cells. Skip fields where there are no cells or bacteria, and do not average these fields in the counts if there are fields where cells and/or bacteria are present.
	3. An acceptable specimen will yield less than 10 squamous epithelial cells (SECs) per low power field.
	4. In general, the ratio must be ≥ 2:1, WBCs to SECs or mucus threads present with <10 cells (PMNs or SECs). The number of WBCs may not be relevant if the patient is neutropenic.
	5. If ≥10 SECs per low power field, report results using the Sunquest code **SALV:** SPECIMEN MICROSCOPICALLY RESEMBLES SALIVA; suggest repeat specimen.
	6. DO NOTreject sputum specimens on CF patients. Rejection on the basis of specimen quality is inappropriate because the organisms associated with CF disease and their presence in culture is significant regardless of the Gram stain findings.
3. Examine the slide under oil immersion (100X).
	1. Quantitate bacterial and fungal morphotypes according to Gram Stain procedure.
	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. **Sputum specimens with abundant epithelial cells and also many WBCs, (1:1 ratio):**

If the Gram stain has 4+ epithelial cells and an area of WBCs with a predominant organism, it is acceptable for culture.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. Vortex specimen until well homogenized if possible.
2. Using a sterile swab, inoculate the media from the purulent portion of the specimen.
3. Roll swab across the upper quadrant of the CHOC, SB, CNA, MAC, PCM, CSA and SABC, touching all surfaces of the swab.
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 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.
7. 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.
8. Make small cuts in the primary area of the SB and CNA for hemolysis.

1. **Incubation**
2. Incubate CHOC, SB, and CNA in 4-10% CO2 at 35ºC
3. Place MAC, PCM, CSA and SABC in ambient air incubator at 35ºC.
4. **Culture examination**
5. Day 1:
6. Examine plates.
7. Gram stain each colony type and perform initial identification procedures, i.e., catalase, oxidase, bile solubility, etc.
8. Correlate colony types with the direct Gram stain.
9. 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. Squamous epithelial cells represent saliva contamination and the isolate work-up should be minimal.
10. Set up definitive biochemical or identification procedures on significant organisms if well isolated. 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. Send updated or final report.
19. MRSA isolation requires a “Called to” if not from E.D. (disch.), or a repeat isolate. Freeze for future reference.
20. 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.
21. Additional Days
22. Complete identification and susceptibility testing procedures until all significant isolates are finished.
23. Hold CF culture plates for a minimum of three days. Hold MAC, SABC, and PCM for 5 days.
24. Send updated report and finalize.
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| **Interpretation** | 1. Sputum cultures with many epithelial cells and few WBCs on Gram stain:
2. If no predominant organism was observed in the Gram stain and the plates have no predominant organism or potential pathogens, the culture does not need to be further evaluated. Report as “**USUAL UPPER RESPIRATORY FLORA**”, code: **UOF.**
3. If a predominant organism was seen on the Gram stain, identify the organism on culture that corresponds to the Gram stain. If the predominant organism is part of UOF, no further identification is necessary.
4. Perform antimicrobial susceptibility testing (AST) **only if requested** otherwise report as “Susceptibilities not performed.” Sunquest code **SNP.**
5. Sputum cultures with few or no epithelial cells:
6. Identify all potential pathogens (PP) regardless of quantity.
7. Perform AST on up to 2 PP if the organism is moderate to predominant.
8. Refer to procedure notes for further information.
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| **Limitations** | 1. A sputum culture has limited value for the diagnosis of pneumonia. Blood cultures are recommended for diagnosis of pneumococcal pneumonia.
2. Do not reject sputum specimens from CF patients based on the Gram stain results. 5-10% of these patients may have difficulty producing an expectorated specimen.
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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*, not predominant Yeast in low numbers (1+)<--non CF patientsBeta-hemolytic streptococcus, group F Small colony β strep (colonies <0.5 mm) Gram-negative rods in low numbers (1+)<-non CF patients only1. 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*Gram negative rods*Enterococcus spp.* 1. Streptococci
2. Alpha-hemolytic (AHS)
* Perform VITEK MS for identification or
* Perform direct bile solubility test or
* Subculture to SB with optochin disk on any predominate alpha hemolytic colonies that resemble *S. pneumoniae.*
* 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. *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—**non CF patient** guidelines---see #11 below for CF guidelines
* 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*
* *Ps. aeruginosa* is a major potential pathogen. Always perform ID and AST.
* *Pasteurella:*
* 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 isolate to the U for M for susceptibility testing.
* 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.

Gram-negative rods—**non CF patient** guidelines* Refer to following chart for work-up of gram-negative rods on non-CF patients.

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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, identification1. *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 | Can this be part of Usual oral flora?? | Identify | Processing level |
| *S. aureus*  | NEVER | Any amount | Perform PBP2a and or AST  |
| *Ps. aeruginosa* (matte and mucoid colony types) | NEVER | Any amount | Perform ID and AST (and AZT KB) |
| Alcaligenes xylosoxidans *(or any other non-fermenter)* | NEVER | Any amount | Perform ID and AST  |
| *Burkholderia cepacia* complex b **Confirm ID with Vitek** | NEVER | Any amount | Perform ID and AST  |
| *S. maltophilia, Burkholderia gladioli, B. bronchiseptica, Pandoraea sp.* and *Ralstonia sp.* | NEVER(Considered emerging pathogens) | Any amount | Perform ID and AST by MicroScan |
| *Enterobacteriaceae sp.* | NEVER | Any amount | Perform ID only; AST upon request |
| *H.* *influenzae* | If Not predominant | Predominant | Perform ID and β-lactamase; Send to MDH for serotype if colony is mucoid or wet |
| *S. pneumoniae* | If Not predominant | Predominant | Perform AST  |
| Mold’s, *Aspergillus* sp. | NEVER | Any amount | Perform presumptive ID only; send to MDH if complete ID requested. Refer for AST upon special request. |
| Rapid growing mycobacteria | NEVER | Any amount | Identify to species level; send to MDH for complete 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
1. *Burkholderia cepacia* complex*:*
* *B. cepacia* will appear pink on PCM.
* Perform Vitek MS for identification.
* Confirm *B. cepacia* identification on Vitek.
* Alert ordering physician.
* Send *B. cepacia* to the University of Michigan reference laboratory and
* Freeze isolate for future reference.

 c. Send Isolates of *B. cepacia, Pandoraea sp. and Ralstonia sp.* to*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 |
| **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 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 procedure for interpretation and resulting.

Observations: 1. 2+ GRAM POSITIVE COCCI1. 4+ WBC'S
2. Review **Culture Summary** for accuracy before filing report.
3. Continued reports: If there are more isolates to report than there are available lines in Sunquest it will be necessary to create a continued report. In Order Entry, order SPCC (Sputum Culture Continued Report using the same date/time of collection. Add “SEEC” to the original accession and “RCON” to the new accession. It will be necessary to free text the new and old accessions after the SEEC and RCON comments. Refer to [MCVI 5.0 Micro Computer Training](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.
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 [MCVI 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.

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.
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| **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. 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.
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| **Appendices** | Work label Definition: BATTERY : SPUC SPEC MEDIA SPCF CHOC, SB, CNA, MAC, PCM, SABC, CSA, GMST, CUT3 SPU CHOC, SB, CNA, MAC, GMST, CUT3 |
| **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 | 02/27/1995 |  |
|  | 1.3 | Pat Ackerman | 07/31/2003 |  |  |  |
| 1.4 | Pat Ackerman | 09/05/2005 |  |
| 1.5 | Pat Ackerman | 08/20/2007 | Sunquest 6.2 reporting information. Revised SRPT and CORR statements. Updated CF traditional organisms in table format. Limit yeast identification to physician request only. |
|  | 1.6 | Tina Gronquist | 06/02/2014 | Updated into online format. |
|  | 1.7 | Becky Carlson | 3/16/2015 | Reworded gram stain acceptability criteria to reflect laboratory practice. |
|  | 2 | Becky Carlson | 4/18/2015  | Re-numbered from MC 426 for CMS load.  |
|  | 3 | Eileen Brinkman Susan DeMeyere | 2/5/2018 | Updated bile solubility testing on alpha strep. |
|  | 4 | Susan DeMeyere | 11/2/2020 | Updated organisms reporting instructions |
|  |  |  |  |  |