Abscess Culture

Purpose

This procedure provides instructions for Abscess Culture in the microbiology laboratory.

Policy Statements

This procedure applies to microbiologists who perform culture set-up and plate reading.

Principle and Clinical Significance

Infections of the subcutaneous tissues may manifest as abscesses, ulcers or boils. Many microorganisms can be associated with these infections, with *Staphylococcus aureus* being the most common in healthy individuals. Many abscesses contain mixed bacteria, which often is dependent on the site of infection. Interpretation of the culture results should be based on the Gram stain criteria and laboratory testing.

Test Code

AC

Materials	Reagents	Supplies	Equipment	Media
	• Gram Stain Reagents	• Glass Slide (GMST) • Anaerobic Gas Pack • Sterile disposable pipette • Sterile tube • Palladox catalysts • Inoculating Loop	• Ambient air incubator • Anaerobic jar • CO_2 incubator • Incinerator • Microscope • Vortex mixer • Anoxomat • Vitek 2XK • Vitek MS	Refer to the Sunquest specimen label for media information. The specimen site determines appropriate media. • Chocolate agar (CHOC) • Sheep Blood Agar (SB) • CNA Agar (CNA) • MacConkey Agar (MAC) • Thioglycolate (THIO) • Sterile saline 1.0mL (SLNE)

Sample

- Acceptable specimens:
  - Pus, fluid or aspirated material.
  - Aspirated material is superior to a swab specimen.
- For additional information: Refer to the Lab Test Directory Abscess Culture for collection and transport instruction.
  - A Sample Rejection section is also available. DO NOT refrigerate specimens.
  - ESwab collection device is acceptable.
  - Refer to MCVI 2.1 Specimen Rejection Criteria

Special Safety Precautions

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies:

- *Biohazard Containment*
- *Safety in the Microbiology Laboratory*
- *Biohazardous Spills*

Procedure

A. Inoculation

1. Allow all media to come to room temperature before inoculation.
2. Label all plates, tubes and slides properly with the patient's name, accession number and date.
3. 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.
4. Always inoculate the culture media first before preparing the slide when using the same pipette.

B. Specimen processing

1. Aspirates and exudates

    a. If specimen is received in a syringe, transfer the entire amount into a sterile tube and mix thoroughly. Vortex on low speed to limit introduction of oxygen.
    b. If the specimen is received in a syringe and the volume is small, rinse syringe with a small amount of THIO or sterile saline (SLNE) to remove the specimen from the syringe. Mix well.
    c. Place 1-2 drops directly on each plate and into a THIO. Place one drop of specimen on a slide for Gram stain.
    d. Spread the specimen on the slide to make a thin film. Poor Gram stain results will occur if the smear is too thick. If the aspirate fluid is clear, use the cytocentrifuge to concentrate the specimen for the smear.

2. Specimens received on swabs

    a. Place the swab into 1.0 ml of sterile saline and vortex.
    b. Squeeze the swab against the side of tube to express remaining fluid and then discard.
    c. Place 1-2 drops directly on each plate and into THIO. Place one drop of specimen on a slide for Gram stain

3. Specimens received on ESwabs

    d. Vortex ESwab.
    e. Place 1-2 drops of liquid media directly on each plate and into THIO. Place one drop of specimen on a slide for Gram stain

4. Streak plates semi-quantitatively for primary isolation.

    a. Sterilize the inoculating loop in the incinerator for 5 to 10 seconds. Allow the loop to cool.
    b. Pass the loop back and forth through the inoculum in the first quadrant several times, covering approximately ¼ of the plate.
    c. 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.
    d. 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. See Figure 1 for an illustrative example.

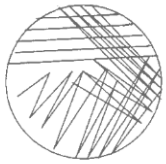

Figure 1. Semi-quantitative plate streaking.

C. Incubation

1. Incubate CHOC, SB, and CNA in 4-10% CO_2 at 35ºC.
2. Incubate MAC and THIO in ambient air incubator at 35ºC.

D. Gram stain examination

1. Perform Gram stain and interpret.
2. Quantitate PMNS, epithelial cells, histiocytes, bacterial and fungal morphotypes.
3. Blot excess oil from slide. Hold slide for one week.
4. If a Gram stain QA failure should occur, review slide and culture. Hold culture plates an additional day if necessary.

E. Culture examination

1. Day #1

   a. Examine aerobic plates and THIO.

   b. Plated media
      1. Gram stain each colony type and perform initial identification procedures (i.e., catalase, oxidase, 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. Epithelial cells represent contamination.
      4. Set up definitive biochemical or identification procedures on significant organisms if well isolated (i.e., VITEK MS, VITEK 2).
      5. Perform antimicrobial susceptibility testing on significant organisms if well isolated.
      6. Subculture organisms that are not well isolated to appropriate media for further work-up.
      7. MRSA isolation requires a “Called to” if not from E.D. (disc), or it is a repeat isolate.
      8. Re-incubate primary plates and subcultures for an additional day.
      9. Report preliminary results.

   c. THIO broth
      1. Visually inspect THIO.
      2. If growth is observed, perform gram stain on THIO.
      3. Correlate the culture result with the Gram stain of the THIO. Do not subculture the THIO if the smear correlates with the growth on the plates. Discard after 2 days.
      4. Subculture THIO’s that are turbid when plates are negative for growth and Gram stain from the THIO is no organisms seen. Subculture to CHOC and SB.
      5. If there appears to be additional organisms in the THIO that are not on the plates, determine if Anaerobic Culture has been ordered.
         - If Anaerobic Culture has **not** been ordered, subculture to appropriate aerobic and anaerobic media. Identify appropriate organisms. Add bill code ANAID.
         - If Anaerobic Culture has been ordered, subculture to appropriate aerobic media. Identify appropriate organisms. If organism in THIO

appears to be an anaerobe, confirm isolation of organism in Anaerobic Culture before finalizing culture.

2. Day #2

   a. Examine primary plates from the previous day for additional microorganisms.
   b. Read and record identification tests and susceptibilities from the previous day.
   c. Set up additional tests as needed.
   d. Visually inspect THIO. If growth is observed, perform gram stain on THIO. Refer to section 'c' above for further instructions.
   e. Ensure THIO with growth was gram stained for 2 consecutive days.
   f. Consult senior techs when the identification or extent of the identification is not clear. Delays in identification can affect patient care.
   g. Send updated report.
   h. If there is no growth on the plates, they can be tossed at 2 days. Culture is held open while THIO continues to incubate.
   i. Hold negative THIO for 5 days. If no growth in THIO, final the report as "No Growth, 5 days".
   j. 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.
   k. Save a representative primary plate for anaerobes in an anaerobic jar or bag, whether a complete work-up was performed or not, at room temperature for 7 days in case a physician calls for further studies.
   l. If the culture is from a sterile body site, or is a significant isolate, save plates for 14 days in red save boxes under DSK5.

3. Additional Days

   a. Complete identification and susceptibility testing procedures until all significant isolates are finished.
   b. Send updated report and finalize.
   c. Send invasive pathogens cultured from sterile sites to MDH for the EIP program. Refer to MCVI 4.1 for MDH Submission or poster on incubator.

Limitations

- Slow-growing *Mycobacterium* sp. or *Nocardia* sp., which may cause abscesses, will **not** be recovered in routine bacterial cultures even if present, since extended incubation periods or special media are necessary for their isolation. Cultures for these organisms should be specifically requested.

Method Performance Specifications

1. Perform definitive identification and susceptibility testing on the following:
   a. Any quantity of a **probable pathogen**, i.e., *S. aureus, Ps. aeruginosa,* etc.
   b. Predominant to moderate numbers of **potential pathogens** with unpredictable susceptibility patterns such as the Enterobacterales.
      - If only one or two species are present or predominant and WBCs are seen on the smear, identify and perform AST.
      - If >2 species are present with no predominant strain, an ID is required. Consult caregiver regarding AST. Perform AST on multiple GNRs only on special request.
   c. Organisms isolated from **patients with catheter-related infections**.
   d. Organisms isolated in **pure culture** and observed in the specimen Gram stain.

2. Because of their known **virulence factors**, report the following:
   a. Beta-hemolytic streptococci
   b. Staphylococcus aureus
   c. Pseudomonas aeruginosa
   d. Identify and report *Clostridium perfringens*, *Bacteroides* sp., *Fusobacterium* sp. if not identified from Anaerobic Culture.

e. If three or more anaerobe species are present, report "Mixed anaerobic flora, no further identification" (**MIXA**).

3. Identify any **yeast** to species level. If yeast in present in small numbers along with mixed flora, no identification is necessary.

4. **Molds**: Identify all molds. Perform Lactophenol cotton blue wet mount. If morphology is consistent with Aspergillus species, report as "presumptive Aspergillus". Send all others to MDH for complete ID.

5. Identify organisms that are **always considered pathogenic**, i.e., *Brucella, Haemophilus, Pasteurella, and Francisella.* These organisms do not grow on MAC.
   - *Francisella* and *Brucella* can be found in lymph node biopsies and are extremely infectious.
   - *Francisella* is a tiny coccobacillus that grows slowly and is catalase positive and oxidase negative.
   - *Brucella* is also a tiny slow growing coccobacillus that is catalase positive, oxidase positive and urease positive.
   - Refer to MCVI 3.60 Bioterrorism Protocol for identification procedures.

6. ***Pasteurella* spp.**
   a. Perform β-lactamase testing. β-lactamase positive isolates are resistant to ampicillin, amoxicillin, and penicillin.
   b. 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.

7. ***Viridans group streptococci* or *enterococci***
   c. Identify at least to the genus level from surgically, invasive specimens.
   d. The anginosis group ("S. milleri") is often associated with brain and liver abscesses. Identify to the species level.
   e. *S. bovis* is associated with gastrointestinal malignancies.

8. **Gram- positive rods**
   a. Rule out *Listeria, Erysipelothrix, Bacillus cereus, Bacillus anthracis, Arcanobacterium,* rapid-growing *Mycobacterium,* and *Actinomyces. B. cereus* has been associated with brain and lung abscesses. *B. cereus* is beta-hemolytic and resistant to penicillin.

9. Perform limited identification and no susceptibility testing on **probable skin contaminants**, which include:

Coagulase-negative staphylococci	diphtheroids
Viridans streptococci	*Bacillus* sp. with many epithelial cells / no PMNS

10. Isolates from sources such as decubitus ulcer, perianal abscess or fistula or intestinal drainage:
    a. If one to two organisms are clearly predominant, perform ID and susceptibility.
    b. Mixed cultures of more than 3 organisms generally represent contamination or colonization.
       - If the culture grows more than 3 organisms, none which is predominant, report as "MIXED FLORA, no further identification" (**MF**), or "MIXED ANAEROBIC FLORA, No further identification" (**MIXA**).
       - Hold plates for further identification.

11. Swabs collected from non-sterile body sites may be contaminated with usual flora from the area. If 3 or more organisms of usual flora is present, you may result as usual flora for the site:
    - **Mouth/throat-**Report **UOF** if resembles normal mouth/throat flora with >3 organisms.
    - **Skin-**Report **USF** with 3 or more skin contaminants.
    - **Perianal/anal**-Report **MF** with 3 or more stool contaminants.

12. Consult with physician on questionable cultures regarding extent of work-ups and susceptibility testing.

13. Emerging infections program (EIP): Send invasive pathogens to MDH cultured from sterile sites.

Invasive Pathogens:	*Neisseria meningitides* *Haemophilus influenzae* Beta *streptococcus* group A Beta *streptococcus* group B *Streptococcus pneumoniae* *Kingella kingae*

Critical Results

Critical results are reported with 60 minutes to the provider. Refer to MCVI 4.0 Critical Results procedure for full list. Organisms to watch for include:

- MRSA
- ESBL or Carbapenemase producers
- Agents of Bioterrorism--*Bacillus anthracis, Brucella, Burkholderia mallei/pseudomallei, Francisella tularensis, or Yersinia pestis*
- Fungal or Yeast isolates:
  - Mucorales-all genera included in this Order, including those listed below
    - *Mucor spp.*
    - *Rhizomucor spp.*
    - *Rhizopus sp.*
    - *Syncephalastrum spp.*
    - *Lichtheimia spp.*
    - *Cunninghamella spp.*
  - *Cryptococcus neoformans*
  - *Coccidioides immitis*
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Sporothrix schenkii*
- VRE
- *Corynebacterium diphtheriae*

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

a. **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 5 days.

b. Positive culture:

Observations: 1. 4+ STAPHYLOCOCCUS AUREUS Further identification to follow

Workups:

Wkup # 1		Workup Components	
Med	: SB	GMS	: STPH
Desc	: BH	SC	: SB
Id	: SAUR	SLC	: POS
		VMIC	: 1

MSID :1

c. If growth is only in the THIO, report as:

Observations: 1. GRAM NEGATIVE RODS ISOLATED FROM BROTH ONLY Further identification to follow (**GNR-BO-FID**)

Workups: Workup # 10 Workup Components
Med : THIO SC : SB MAC
Desc : CLDY GMS : GMNR
ID : GNR

d. 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

e. Review **Culture Summary** for accuracy before filing report.

2. MRSA isolation requires a "Called to" if not from E.D. (disc), or a repeat isolate. Document date and time called in computer. Freeze for future reference.

1. 3+ METHICILLIN-RESISTANT STAPH AUREUS ***MDRO***
2. MULTIPLE DRUG RESISTANT ORGANSIM (MDRO): This organism requires SPECIAL CONTACT PRECAUTIONS. Please call Infection Control.
3. **Called to Linda S., RN L8 @ 1300 7/7/03

3. If 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.

4. Continued reports: If there are more isolates to report than lines in Sunquest it will be necessary to create a continued report. In Order Entry, order ACC (Abscess Culture Continued Report), using the same date/time. 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 Specimen Ordering and Receiving for complete details.

5. 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 Mislabeled & Unlabeled and Correcting Patient Data.

References

1. Leber, Amy. Clinical Microbiology Procedures Handbook, 4th edition. Vol. 1-3. 2016. American Society for Microbiology, Washington D.C., 20036.
2. *LRN Level A Bioterrorism Laboratory Protocols,* 2013, Minnesota Laboratory System
3. Clinical and Laboratory Standards Institute CLSI M45 3rd edition 2015

Training Plan	Initial Competency Assessment

Training Plan/ 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.

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Pat Ackerman	1978	Initial Version
1.1	Pat Ackerman	01/1992	
1.2	Pat Ackerman	07/05/2003	
1.3	Pat Ackerman	07/27/2004	
1.4	Pat Ackerman	06/02/2007	Updated Sunquest 6.2 reporting information. Revised SRPT and CORR statements.
1.5		01/14/2008	Added EIP information.
1.6	Jessica Craig	05/20/2010	Updated into online format.
1.7	Jennifer Johnson	03/04/2013	Removed MRSA to MDH; removed CAID testing for yeast
1.8	Becky Carlson	12/30/2013	Added AST consultation. Save policy: sterile body site and significant isolates for 14 days.
2	Becky Carlson	4/10/2015	Renumbered from MC 401. Re-formatted for CMS.
3	Susan DeMeyere	9/1/2017	Changed reporting to keep culture open while THIO is incubating.
3	Susan DeMeyere	10/22/2018	Biennial Review
4	Susan DeMeyere	10/31/2018	Removed culturing for anaerobes on initial set up. Added instructions for THIO processing.
5	Susan DeMeyere	10/16/2020	Removed use of Scant when growth only from THIO. Added instructions for *Pasteurella*.
6	Susan DeMeyere	11/6/2024	Added THIO instructions. Added swab from non-sterile site instructions.