PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Wound, Abscess, and Soft Tissue Culture Procedure

Department of Microbiology

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1.0 Clinical Significance

A wide variety of microorganisms that reside on the skin and mucous membranes of the body, as well as those found in the environment, can cause skin and soft tissue infections. These organisms enter through breaks in the skin or mucous membranes, through wounds made by trauma or bites (exogenous) or as a complication of surgery or foreign-body implants (endogeneous) or they can spread through the vascular system (hematogenous).

Acute wound infections are normally caused by external damage to intact skin, such as those produced during surgery or by trauma and bites. Conversely, chronic infections, such as decubiti or foot and leg ulcers, are normally due to complications related to impaired vascular flow or metabolic disease (e.g., diabetes mellitus). Wound colonization and/or infection is often polymicrobic, with both aerobes and anaerobes involved.

2.0 Principle

An aspirate obtained through intact skin by needle and syringe is the best type of specimen to obtain for culture. If the skin surface and surgical areas are properly disinfected prior to specimen collection, the organisms present can be assumed to be the cause of infection. Interpretation of microbial cultures taken from open skin or abscesses may be compromised, due to the fact that these lesions are often colonized with a large number of indigenous microbiota. Such cultures are indicated only if there are clear signs of infection or if a wound is failing to heal. Proper preparation of the wound prior to specimen collection can minimize contamination. After appropriate debridement and cleansing of the wound, the specimen should be obtained by biopsy of the leading edge of the lesions, where the pathogens should be present and colonizing bacteria are less likely to occur. Bacterial cultures of purulent material obtained by needle and syringe aspiration can also provide meaningful results. If an aspirate or tissue sample cannot be obtained, swab collection of exudate from the deep portion of lesions can be submitted. Swabs are the least appropriate collection method for culture, as the organisms isolated may only be colonizing the area and not involved in the infective process.

The primary agents of skin and tissue infections are *S. aureus, P. aeruginosa,* members of the *Enterobacteriaceae,* beta-hemolytic streptococci, and a variety of anaerobes. In appropriately collected specimens, the presence of one of these organisms may indicate the need for antimicrobial therapy. Since wound infections can be polymicrobic, treatment may need to be broad spectrum, and there is little need to perform ID/AST on all isolates.

The accumulation of inflammatory cells and the resultant collection of pus within an abscess or a sinus tract is a hallmark of local skin infection. Evidence of this process can be documented by the presence of neutrophils in the Gram-stained smear. Therefore, the quality of a wound specimen can be assessed by Gram stain, which should be used to guide the extent of the culture workup. The presence of epithelial cells indicates contamination of the specimen with skin or mucous membrane microbiota and may compromise the significance of the culture results. However, the presence of organisms in the Gram stain of an appropriately collected specimen from an infected wound correlates with a clinically significant isolate in culture.

3.0 Scope

This procedure is classified under CLIA as highly complex. It should be carried out by technical personnel familiarized and trained to differentiate and identify potential pathogens. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and antimicrobial susceptibility testing.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used for isolate identification may be hazardous to your health if handled incorrectly. Information concerning the safe handling of the reagents and/or

chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves must be worn when handling specimens.
- A laboratory coat must be worn when handling specimens, cultures, and reagents.
- A biological safety cabinet must be used when processing specimens. For cultures of lymph nodes, work in a biological safety cabinet, since pathogens can be found in these specimens that are hazardous, e.g., *Brucella, Francisella,* and *Mycobacterium.*

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Collection, Handling, and Storage

5.1 General Considerations

1. Cleanse skin or mucosal surfaces.
    - For closed wounds and aspirates, disinfect as for blood culture collection. Remove disinfectant with alcohol prior to collection.
    - For open wounds, debride, if appropriate, and thoroughly rinse with sterile saline prior to collection.
2. Sample viable infected tissue, rather than superficial debris.
3. Avoid swab collection if aspirates or biopsy samples can be obtained.

5.2 Swabs of Wounds

1. After proper disinfection and cleaning, gently roll swab over the surface of the wound approximately 5 times, focusing on the area where there is evidence of pus or inflamed tissue.
2. Place the swab in a device containing transport medium and label the container.

5.3 Abscesses

1. Aspirate infected material with a needle and syringe.
2. Place purulent material in a Vacutainer tube without anticoagulant and label the container.

5.4 Storage and Transport

Ideally, aspirates should be delivered to the laboratory within 30 min for best recovery of microorganisms. If there is a delay, keep the sample at room temperature, because at lower temperatures there is likely to be more dissolved oxygen, which could be detrimental to anaerobes. Swab samples submitted in transport medium can be sent at room temperature or refrigerated for up to 1 d.

5.5 Rejection Criteria

1. Do not accept any specimen submitted in formalin.
2. Syringes with the needle attached should not be accepted due to the sharps/biohazard risk. Syringes with Leur-Lok are suboptimal as they may leak during transport and the sample may get contaminated during handling.

3. For multiple requests (AFB, fungus, routine bacterial) with little sample, contact the physician to determine which assays are most important.

6.0 Materials

6.1 Equipment

- CO_2 incubator set at 35 ± 2°C
- Anoxomat system and jar
- Automated streaking instrument
- Microscope with 10× and 100× objectives

6.2 Consumables

- Sterile disposable loops (if streaking by hand)
- Glass microscope slides

6.3 Media

- 5% Sheep Blood agar (BAP)
- Chocolate agar (CHOC)
- MacConkey agar (MAC)
- CNA agar (CNA)

7.0 Specimen Processing

7.1 Aspirates and Pus

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Mix the specimen thoroughly.
3. Prepare smear for Gram stain by placing a drop of specimen on a slide and spreading it to make a thin preparation.
4. Place a drop of specimen onto each piece of medium.
5. Quadrant-streak each plate for isolation.

7.2 Spun-fiber Swabs

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Prepare smear for Gram stain by pressing and rolling the swab in the center of a glass slide to express absorbed material.
3. Use the swab to directly inoculate the media. Press and roll the swab against the surface of the agar in the first quadrant. Inoculate the least inhibitory media first (BAP & CHOC).
4. Quadrant-streak each plate for isolation.

7.3 Flocked Swabs in Liquid Amies (ESwab)

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Vigorously shake the ESwab tube containing the swab sample between the thumb and forefinger for 5 s or mix the tube using a vortex for 5 s to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid transport medium.
3. Unscrew the ESwab cap and remove the swab applicator.
4. Transfer 1 to 3 drops of the suspension onto each culture plate using a sterile pipette.
5. Prepare a smear for Gram stain by transferring 1 or 2 drops of the suspension onto the center of a glass microscope slide. In case of bloody or thicker specimens particular care should be taken to thinly spread the sample on the slide. Place the slide in an electric slide warmer set at a temperature not exceeding 42°C.
6. Fix smears using methanol for 1 min and allow the smear to air dry. Methanol fixation is recommended as it prevents lysis of red blood cells, avoids damage to all host cells, results in a cleaner background, and prevents liquid specimens from washing off.

7.4 Incubation

1. Incubate the BAP, CHOC (if applicable), CNA, and MAC in a humidified incubator at 35 to 37°C with 5% CO_2.
2. Incubate the BAP-ANA plate in an Anoxomat jar using the anaerobic recipe on the processor.

8.0 Culture Workup & Reporting

Caution: *For cultures of lymph nodes, work in a biological safety cabinet, since pathogens can be found in these specimens that are hazardous, e.g., Brucella, Francisella, and Mycobacterium.*

8.1 Potential Pathogens

Any organism is considered a potential pathogen if seen in the direct Gram stain. The most common pathogenic organisms associated with wound infections are:

- *Staphylococcus aureus*
- Beta-hemolytic streptococci
- *Enterococcus*
- Gram-negative rods
- Anaerobic bacteria
- Yeast

8.2 Mixed Flora from Non-sterile Body Sites (other than oropharyngeal)

Mixed flora includes any of the following organisms from non-sterile body sites other than oropharyngeal sites (see note below concerning exceptions). Unless seen in the direct Gram stain, these organisms are reported as "mixed flora" with no additional workup or AST.

- *Bacillus* spp.
- Coagulase-negative *Staphylococcus* spp.
- *Corynebacterium* spp.
- *Streptococcus* spp. (other than beta-hemolytic strep or *Enterococcus*)
- *Micrococcus* spp.

Note: These organisms may be involved in opportunistic infections in patients that have undergone an invasive procedure (e.g., sternum incision). Normally, the significance of these isolates in culture is determined by whether or not they are seen in the direct smear. However, there may be specific cases where a singular morphotype grows and may represent a significant isolate even though it was not seen in the direct smear. These isolates may warrant identification and susceptibility testing. Consult Rounds.

8.3 Mixed Flora from Non-sterile Face, Mouth, Jaw, or Neck Specimens

Mixed flora includes any of the following organisms from non-sterile face, mouth, jaw, or neck specimens. Unless seen in the direct Gram stain, these organisms are reported as "mixed flora" with no additional workup or AST.

- *Fusobacterium* spp.
- *Peptostreptococcus* spp.
- *Neisseria* spp.
- Coagulase-negative *Staphylococcus* spp.
- *Streptococcus* spp. (other than beta-hemolytic strep or *Enterococcus*)
- Corynebacterium spp.

8.4 Day 1

After at least 18 to 24 h of incubation, examine the plates for growth. Anaerobic plates that do not have growth distinctive from the aerobic plates should be immediately returned to anaerobic conditions. Note the quality of the specimen (Q score) and the organisms that were reported in the Gram stain. Determine the number of potential pathogens growing in the culture (exclude bacteria included as mixed flora in sections 9.2 and 9.3).

8.4.1 Sterile Cultures

1. Report: **No growth to date**.
2. Re-incubate the plates.

8.4.2 Potential Pathogens ≤ Q Score

1. Speciate and report the presumptive isolate(s) according to the identification charts, and document workup in the computer.
2. Perform susceptibility testing on organisms, if appropriate.
3. Re-incubate the plates.

8.4.3 Potential Pathogens > Q Score, but Correlating Pathogens are < Q Score

1. If the correlating potential pathogens do not exceed the score, speciate and report the presumptive isolate(s) according to the identification charts, and document workup in the computer.
2. Perform susceptibility testing on correlating organisms.
3. Report non-correlating isolates generically by reporting as: **Mixed flora including (list non-correlating potential pathogens)**.
4. Attach the comment: **This is a mixed culture of potential pathogens. Correlation of the culture results with the gram stained direct smear indicates one or more isolate is more significant than others. The organisms seen only in culture may not relate to infection and may represent colonization or contamination. [MXSIG]**
5. Re-incubate the plates.

Note: The following organisms should be identified and reported, regardless of correlation with the Gram stain or mixture of organisms.

- *Identify and report any S. aureus with AST results.*
- *Identify and report any beta-hemolytic Group A Strep along with the canned susceptibility comment [BSAS].*

8.4.4 Potential Pathogens that Correlate > Q Score

1. Report the isolates generically: **Mixed flora including (list potential pathogens)**.
2. Attach the comment: **This is a mixed culture of potential pathogens. Correlation of culture results with the gram stained direct smear does not identify any isolate as more significant than another. Bacteria may not relate to infection and may represent colonization or contamination. [MXNSIG]**

Note: The following organisms should be identified and reported separately from the list of other potential pathogens, regardless of correlation with the Gram stain or mixture of organisms.

- *Identify and report any S. aureus with AST results.*
- *Identify and report any beta-hemolytic Group A Strep along with the canned susceptibility comment [BSAS].*

8.4.5 Q Score is 0

1. Report potential pathogens generically: **Mixed flora including (list potential pathogens)**. Identification testing should be limited to tests that can be completed on that same day (e.g., gram stain, motility, spot tests, etc.).
2. After listing potential pathogens, add the comment: **This is a mixed culture suggesting the probability of contamination. Collection of another specimen is suggested, avoiding superficial sources of contamination. [SWCONT]**

Note: The following organisms should be identified and reported separately from the list of other potential pathogens:

- *Identify and report any S. aureus with AST results.*
- *Identify and report any beta-hemolytic Group A Strep along with the canned susceptibility comment [BSAS].*

- *Identify and report P. aeruginosa from ear specimens if present in pure culture or with mixed flora. Perform AST. However, if P. aeruginosa is present with other potential pathogens, include in generic list and do not perform AST.*

8.5 Day 2

After 2 d of incubation, examine the plates for growth.

8.5.1 Sterile Cultures

1. If the plates are sterile, report: **No growth.**
2. Discard the cultures of non-sterile sites.
3. Continue to incubate the anaerobic BAP and the CHOC plate for surgical specimens and specimens from sterile sites for 5 days. Enter the following comment: **Plates will be incubated for 5 days. [PL5]**

8.5.2 Culture with Growth on Day 2

Refer to the workup protocol listed under Day1.

9.0 Retention of Cultures

All plates with growth should be held in the storage cupboard for 7 d. Cultures are retained in case the clinician requests additional workup or antimicrobial susceptibility testing.

10.0 Quality Control & Quality Assurance

10.1 Quality Control

1. Verify that media meet expiration date and QC parameters. Each new lot/shipment of CHOC should be tested with control strains (refer to the Quality Control Reference Guide).
2. Temperatures of all controlled instruments and environments must be checked daily and recorded in LIS. This includes refrigerators, freezers, incubators, water baths and heating blocks. The thermometers used for measuring temperatures are checked against a NIST certified thermometer prior to being placed into use and must not vary more than $\pm$ 1° C.
3. Each anaerobic jar should be checked with a methylene blue strip. Before opening each jar, verify that the strip turned white. Document the acceptability of QC for all opened jars in LIS once per shift. CO_2 incubators must be checked daily by the digital reading and weekly using a Fyrite device. Document CO_2 readings in LIS.

10.2 Quality Assurance

Each week the Microbiology director and technical specialist randomly review six wound smears and six wound cultures. Interpretations are compared to the results reported by technical staff in the LIS. If discrepancies are discovered, the reports are corrected or amended and the smear or culture is returned to the original reader for review.

11.0 Limitations

1. The presence of neutrophils is an indication of an inflammatory or infectious process, while the presence of epithelial cells indicates surface contamination of the specimen. Specimens containing numerous squamous epithelial cells yield culture results of questionable accuracy in the diagnosis of the infectious process.
2. If a patient is immunocompromised or has poor vascular supply, inflammatory cells may not be present in the specimen. However, up to three potential pathogens will be worked up even in the absence of neutrophils, provided no squamous epithelial cells are present.
3. Low levels of organisms or fastidious organisms that grow poorly on the direct plates may be missed in culture.
4. Other inflammatory diseases can have the same presentations as infectious diseases.

12.0 References

1. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

13.0 Document Control History

Medical Director Approval: Dr. Schappert 3/10/2010.

Microbiology Director Approval: Dr. Ann Robinson 02/20/2006, 04/27/2006

Microbiology Supervisor Reviews: Jerry Claridge 02/20/2006, 01/2007, 09/2007, 09/2008, 09/2009, 03/2011, 03/2013

Revisions & Updates:

10/27/2010 Added Q0 P. aeruginosa ear workup per AR.

11/08/2012 Added note for surgical specimens under mixed flora.

12/17/14 Updated for working up *S. aureus* regardless of Q score or mixture of organisms. Removed *C. jeikium* from list of potential pathogens (*Corynebacterium* isolates are worked up if seen in the smear). Added work-up of lymph node cultures in a BSC. Added procedure sections: Clinical Significance, Principle, Scope, Safety, Specimen Collection, Materials, Specimen Processing, QA & QC, and Limitations.