PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Tissue Culture Procedure

Department of Microbiology

Effective date: 05/08/2006

Last Revision: 01/13/2015

Last reviewed: 05/2015

Table of Contents

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 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 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). Colonization and/or infection is often polymicrobic, with both aerobes and anaerobes involved.

2.0 Principle

Interpretation of microbial cultures taken from tissue near open skin or mucosal surfaces may be compromised, due to the fact that these lesions are often colonized with a large number of indigenous microbiota. Proper preparation of the infected site prior to specimen collection can minimize contamination. After appropriate debridement and cleansing, 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. If surgical areas are properly disinfected prior to specimen collection, the organisms present can be assumed to be the cause of infection.

Organisms recovered from tissue specimens obtained from closed, normally sterile body sites represent significant, rather than colonizing microorganisms. However, these infections can be polymicrobic and treatment may need to be broad spectrum rather than targeted at specific organisms. Therefore, there is little need to perform ID/AST on all isolates. Typically, up to three isolates would be worked up.

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:

- Airborne and bloodborne 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 culture inoculation and smear preparation. For cultures of lymph nodes growing organisms, 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

Tissue biopsy samples should be collected from areas within and adjacent to the area of infection. Large tissue samples should be collected to perform all of the tests required (i.e., 3- to 4-mm biopsy.

5.2 Tissue for Quantitative Culture

Submit 2- by 1-cm or larger tissue sample collected after cleansing and/or surgical debridement. This will yield approximately 500 mg of tissue, depending on density.

5.3 Storage and Transport

Ideally, tissues should be delivered to the laboratory within 30 min for best recovery of microorganisms. Tissue biopsies should be placed into a sterile container for transport.

1. Keep tissues moist to preserve organism viability. If there is a delay in transport, or if the tissue sample is small, a piece of sterile gauze and sterile saline should be added to maintain the sample.
2. 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.

5.4 Rejection Criteria

1. Do not accept any specimen submitted in formalin.
2. For multiple requests (AFB, fungus, routine bacterial) with little sample, contact the physician to determine which assays are most important.
3. If insufficient specimen is received for quantitative culture, process the specimen for routine qualitative culture only.

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
- Disposable, plastic tissue grinding bag (Whirl-Pak)
- Sterile scalpel
- Sterile petri dish

6.3 Media

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

7.0 Specimen Processing

7.1 Tissue Homogenization

1. Label appropriate media and a glass microscope slide with the test barcode labels.
2. Work with specimen and culture materials within a biosafety cabinet.

3. Select a portion of the tissue biopsy sample for culture that is bordering and within the area of infection. Necrotic tissue may be at the center of infected tissue that was submitted.
4. If tissue is more than 1 cm^3, use sterile forceps and scalpel to cut into sections.
5. Use cut tissue to make touch preps for smears. Place the slide on an electric slide warmer set at a temperature not exceeding 42°C.
6. Place tissue in a Whirl-Pak bag with up to 0.5 mL of sterile saline. Expel as much air as possible and seal bag by folding the top of the bag down several times. Place inside of a second Whirl-Pak. Roll a thick marking pen over bag until tissue is dispersed and homogenized in the saline. Open the bag and use a sterile pipette to inoculate the homogenized material to appropriate media.
7. Quadrant-streak each plate for isolation.
8. Save the specimen in the bag, along with leftover tissue, at -70°C.

7.2 Tissue for Quantitative Culture

1. Weigh the tube containing the tissue on an analytical balance.
2. Remove the tissue by using aseptic technique, and place it in 5 mL of sterile 0.85% saline in a Whirl-Pak. This is approximately a 1:5 dilution of tissue.
3. Reweigh the now empty original specimen tube, and subtract to determine the weight of the tissue in mg.
4. Homogenize the tissue as described above.
5. Plate 0.1 mL of the homogenate onto each piece of media. Label the plates 10^{-1} for amount of dilution of original homogenate.
6. Make three serial 1:10 dilutions of the homogenate with 0.5 mL aliquots and 4.5 mL of sterile 0.85% saline.
7. Plate 0.1 mL of each dilution onto each piece of media and distribute the inoculum over the surface of each plate using a sterile loop. Label the plates 10^{-2}, 10^{-3}, and 10^{-4}.

7.3 Incubation

1. Incubate the BAP, CHOC, CNA, and MAC in a humidified incubator at 35 ± 2°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. However, tissues obtained from superficial sites or near mucosal surfaces may be contaminated with endogenous flora. Cultures of specimens from superficial or mucosal surfaces that grow a mixture of resident flora should be reviewed during Rounds prior to work-up.

8.2 Qualitative Tissue Cultures – 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. Determine the number of isolates growing in the culture.

8.2.1 Sterile Cultures

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

8.2.2 Growth of ≤ 3 Isolates

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.

8.2.3 Growth of > 3 Isolates

1. Present culture during Rounds prior to work-up.

8.3 Qualitative Tissue Cultures – Day 2

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

8.3.1 Sterile Cultures

1. If the plates are sterile, report: **No growth.**
2. 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]**

8.3.2 Culture with Growth on Day 2

Refer to the workup protocol listed under Day1.

8.4 Quantitative Tissue Cultures – 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.

8.4.1 Sterile Cultures

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

8.4.2 Cultures with Growth

1. Determine the number of organisms per gram of tissue by counting the colonies on the plate that grew between 30 and 300 colonies. Only use the MAC and CNA plates if there is an overgrowth of mixed organisms that prevents accurate counting of colonies.
2. Calculate the total

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.

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 isolates 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.
2. Package insert: Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System, Rev. 04, 10/2010.

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, Jason Ammons 05/2015

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. 01/13/2015 Removed “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.” Information moved to new procedure for ear cultures.