3.13 Wound/Abscess and Soft Tissue Cultures

3.13.1 Wound Cultures

PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE
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 the body 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 (endogenous), or they can be spread to the tissues through the vascular system (hematogenous).

Laboratories may distinguish superficial wound, abscess/fluid, or tissue specimens from those collected from deep body sites (see Table 3.13.1–1). Superficial wound and abscess specimens usually grow primary pathogens causing skin and soft tissue infections. The primary agents of skin and tissue infections are Staphylococcus aureus, Pseudomonas aeruginosa, members of the Enterobacteriaceae, beta-hemolytic streptococci, and anaerobes. A much broader microbial diversity can usually be recovered from deep wound and invasively collected abscess/ fluid and tissue specimens if the appropriate media and cultures are inoculated (see Tables 3.13.1–2 and 3.13.1–3). Table 2.1–1 also summarizes common skin and soft tissue infection diagnoses and their associated pathogens by site.

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 polymicrobial, with both aerobes and anaerobes (see section 4) involved.

Tissues collected during surgery or aspirates obtained through intact skin by needle and syringe or by fine-needle biopsy are the best types of specimen to obtain for microbiology 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 microflora. 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 from the leading edge of the lesion, where pathogens should be present and colonizing organisms 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 collections of exudate from the deep portion of lesions can be submitted. Swabs are the least appropriate specimen for microbiology analysis, because the organisms isolated may only be colonizing the area and not involved in the infective process.

In appropriately collected specimens, the presence of one of the primary agents of skin and tissue infections may indicate the need for antimicrobial therapy. Since skin and soft tissue infections and abscesses can be polymicrobial, empiric treatment is often broad in spectrum, and there is little need to identify and perform antimicrobial susceptibility testing (AST) on all isolates. Tissues and aspirates are acceptable for anaerobic culture, as anaerobes can account for 38 to 48% of the total number of microbial isolates in wound specimens (Table 3.13.1–2 and reference 1). It must be emphasized that wound specimens collected on swabs will be less appropriate than tissues or aspirates for anaerobic culture, provided that the tissues and aspirates are submitted under anaerobic transport conditions.

The accumulation of inflammatory cells and the resultant collection of pus within an abscess or a sinus tract is a hallmark of local infection. Evidence of this process can be documented by the presence of PMNs 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 microbiology testing. 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. Quantitative cultures of tissue specimens have been shown to be useful in evaluation of wound healing related to skin grafting (procedure 3.13.2); however, the presence of organisms in the Gram stain of an appropriately collected specimen from an infected wound correlates with a clinically significant count of bacteria (2). In addition, many publications have shown that, for acute wounds, a swab culture with enumeration of the organisms present correlates well with quantitative tissue cultures.
Table 3.13.1–1  Common types of superficial and deep wounds and abscesses

<table>
<thead>
<tr>
<th>Specimen types</th>
<th>Site or source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td>Boils, furuncles, infected cysts, skin abscesses, superficial surgical wounds</td>
<td>Defined as an infected space that may drain through the skin but does not extend deeper than the dermis; aerobic culture only required.</td>
</tr>
<tr>
<td></td>
<td>Any site, including deep tissues; usually related to secondary infection of a deep wound, or due to hematogenous spread of organisms.</td>
<td>Defined as a closed infected space that extends deeper than the dermis into deep tissues whose cavity may be encapsulated; aerobic and anaerobic cultures required.</td>
</tr>
<tr>
<td>Wound</td>
<td>Abrasion, cut, laceration, or ulcer (any site), plus associated skin diseases (impetigo, folliculitis, cellulites) or burns</td>
<td>Defined as a wound in the skin that does not extend deeper than the dermis; aerobic culture only required.</td>
</tr>
<tr>
<td></td>
<td>Typically applies to deep surgical wounds that go across a mucosal surface (e.g., abdominal, pelvic, or chest), bite wounds, deep traumatic wounds (e.g., gunshots, stabs, punctures); third-degree burns due to electrocution</td>
<td>Defined as a wound that penetrates deeper than the dermis of the skin or is located in deep tissues; aerobic and anaerobic cultures required.</td>
</tr>
</tbody>
</table>

Table 3.13.1–2  Aerobic and anaerobic isolates from acute and chronic skin and soft tissue infections

<table>
<thead>
<tr>
<th>Aerobic and facultative microorganisms</th>
<th>Anaerobic bacteria</th>
<th>Aerobic microorganisms from unusual, specialized, and zoonotic infections</th>
<th>Yeasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>Finegoldia magna</td>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Peptostreptococcus spp.</td>
<td>Aeromonas spp.</td>
<td>Candida krusei</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>Peptostreptococcus spp.</td>
<td>Bacillus anthracis</td>
<td>Candida parapsilosis</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>Actinomyces spp.</td>
<td>Bergeyella zoohelcum</td>
<td></td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>Clostridium spp.</td>
<td>Capnocytophaga spp.</td>
<td></td>
</tr>
<tr>
<td>Arcanobacterium hemolyticum</td>
<td>Eggerthella spp.</td>
<td>Neisseria animoralis</td>
<td></td>
</tr>
<tr>
<td>Corynebacterium spp. b</td>
<td>Eubacterium limosum</td>
<td>N. zoodegmatis</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Propionibacterium acnes</td>
<td>Chromobacterium violaceum</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Bacteroides fragilis group</td>
<td>Eikenella corrodens</td>
<td></td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>Prevotella spp.</td>
<td>Francisella tularens</td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>Porphyromonas asaccharolytica</td>
<td>Haemophilus spp.</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>Fusobacterium necrophorum</td>
<td>Kingella kingae</td>
<td></td>
</tr>
<tr>
<td>Morganella morganii</td>
<td></td>
<td>CDC group NO-1</td>
<td></td>
</tr>
<tr>
<td>Providencia stuartii</td>
<td></td>
<td>Pasteurella multocida</td>
<td></td>
</tr>
<tr>
<td>Proteus spp.</td>
<td></td>
<td>Streptobacillus moniliformis</td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td></td>
<td>Vibrio vulnificus</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingobacterium multivoram</td>
<td></td>
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</tr>
</tbody>
</table>

* Data revised from reference 1.

* Corynebacterium diphtheriae causes skin ulceration and should be ruled when Corynebacterium spp. are isolated in this clinical context.

(see review by Bowler et al. [1] and refer to procedure 3.13.2 for quantitative methods). This section contains information presented in procedure 3.13.1 in the third edition of this handbook (3).
### Table 3.13.1–3
Commonly encountered superficial and deep-wound/abscess, drainage, and tissue infections

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Location of infection</th>
<th>Primary bacterial pathogen(s)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superficial infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boils/carbuncles</td>
<td>Skin and superficial tissues</td>
<td><em>S. aureus</em>/<em>MRSA</em></td>
<td><em>B. anthracis</em> may cause a skin eschar.</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>above the deep fascia and muscle anywhere on the body</td>
<td>Beta-hemolytic streptococci (A, B, C, G)</td>
<td></td>
</tr>
<tr>
<td>Cysts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folliculitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury (abrasions, first-degree burns, cuts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous abscesses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deep-wound infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bites—human</td>
<td>Skin and deeper tissues below the dermis, fascia, and muscle</td>
<td>Animal bites</td>
<td><em>Aeromonas</em> spp., <em>Vibrio</em> spp., or <em>C. violaceum</em> may be found in wounds exposed to water. Anaerobic culture should be performed on request or when the Gram stain suggests the presence of anaerobes.</td>
</tr>
<tr>
<td>Bites—animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second- or third-degree burns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Episiotomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical wounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer (decubiti, diabetic foot, etc.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Abscesses/fluids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirated from deep body spaces or tissues</td>
<td>Mixed aerobes, anaerobes and facultative organisms</td>
<td>Anaerobic culture should be performed on request or when the Gram stain suggests the presence of anaerobes.</td>
<td></td>
</tr>
<tr>
<td><strong>Drainages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid freshly aspirated from disinfected tubing or area being drained</td>
<td>Mixed aerobes, anaerobes, and facultative organisms</td>
<td>Anaerobic culture should be performed on request or when the Gram stain suggests the presence of anaerobes.</td>
<td></td>
</tr>
<tr>
<td><strong>Tissues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasively collected samples of deep organs and tissues</td>
<td>Mixed aerobes, anaerobes, and facultative organisms</td>
<td>Anaerobic culture should be performed on request or when the Gram stain suggests the presence of anaerobes.</td>
<td></td>
</tr>
</tbody>
</table>

*See Table 3.13.1–2 for a complete listing of aerobic and anaerobic isolates from acute and chronic wound/abscess and tissue infections.*

*See procedure 3.5 and Table 3.5–2 for drainages that are acceptable for culture.*

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**II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING**

>*NOTE:* Refer to procedure 3.3.1 for additional details. The following can be copied for preparation of a specimen collection instruction manual for caregivers. It is the responsibility of the laboratory director or designee to educate physicians and other caregivers on proper wound specimen collection.

**A. General considerations**

1. Preferably collect specimen prior to initiation of therapy and only from wounds that are clinically infected or deteriorating or that fail to heal over a long period.
II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING
(continued)

2. Cleanse skin or mucosal surfaces.
   a. For closed wounds and aspirates, disinfect as for a blood culture collection with 2% chlorhexidine or 70% alcohol followed by an iodine solution (1 to 2% tincture of iodine or a 10% solution of povidone-iodine [1% free iodine]). Remove iodine with alcohol prior to specimen collection.
   b. For open wounds, debride, if appropriate, and thoroughly rinse with sterile saline prior to collection.
3. Sample viable infected tissue, rather than superficial debris.
4. Avoid swab collection if aspirates or biopsy samples can be obtained.
5. Containers
   a. Anaerobe transport vial for small tissues
   b. Sterile cup for large tissues with nonbacteriostatic saline on a gauze pad to keep moist
   c. Wound or abscess aspirates
      (1) Samples collected by using a syringe and needle should be placed in a sterile container or blood collection tube without anticoagulant (e.g., Vacutainer or similar type) for submission to the laboratory.
      (2) A portion of the samples should also be placed in a sterile tube containing prereduced anaerobically sterilized (PRAS) medium if an anaerobic culture is required.
      (3) Syringes with the needle attached should not be accepted due to the sharps and biohazard risk to staff. Syringes capped with a Leur-Lok are also not acceptable because the specimen may leak during transport and the samples may also be contaminated during handling.
   d. Broth culture medium in small sterile snap-top microcentrifuge tubes for fine-needle aspirates (FNA). These tubes are ideal for this type of specimen, because the specimen is easily visible and can be minced with a sterile glass rod in the laboratory, if necessary.
   e. Routine culture swabs should be submitted in a transport system with Stuart’s or Amies medium to preserve the specimen and keep it moist and to neutralize inhibitory effects of swabs. CultureSwab EZ II (BD Diagnostic Systems) and ESwabs (flocked swab in 1 ml of liquid Amies medium; Copan Diagnostics) are best for recovery of aerobes and anaerobes using a single specimen collection device (4, 5; http://www.copanusa.com).

B. Specimen collection after proper disinfection
   ☑ NOTE: Refer to procedure 3.13.2 for quantitative culture methods.
1. Swabs of wounds
   ☑ NOTE: Superficial wounds must often be swabbed to collect a sample for culture because there is not enough pus or fluid to aspirate.
   a. Limit swab sampling to wounds that are clinically infected or those that are chronic and not healing.
   b. Superficial or deep wounds, including bites, should be cultured only if there is purulence, chronic drainage, or nonhealing.
   c. Gently roll swab over the surface of the wound approximately five times, focusing on the area where there is evidence of pus or inflamed tissue.
   d. Aerobic and anaerobic culture is indicated for all types of deep wounds and bites that are clinically infected. CultureSwab EZ II (BD Diagnostic Systems) and ESwabs (flocked swab in 1 ml of liquid Amies medium; Copan Diagnostics) are best for recovery of aerobes and anaerobes using a single specimen collection device (4, 5; http://www.copanusa.com). Alternatively, separate swabs must be collected for aerobic and anaerobic culture and immediately placed after inoculation back into their respective transport tubes.
II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING (continued)

Wound/Abscess and Soft Tissue Cultures

3.13.1.5

NOTE: Organisms may not be distributed evenly in a burn wound, so sampling different areas of the burn is recommended. Blood cultures should also be used to monitor the septic patient’s status.

2. Abscesses

NOTE: Abscesses (purulent collections) that are closed off and not yet draining externally should be aspirated and the pus (i.e., purulent fluid) sent for culture.

a. Aspirate infected material with needle and syringe.

b. If the initial aspiration fails to obtain material, inject sterile, nonbacteriostatic saline subcutaneously. Repeat the aspiration attempt.

c. Wound or abscess aspirates collected by using a syringe and needle should be placed in a sterile container or blood collection tube without anticoagulant (e.g., Vacutainer or similar type) for submission to the laboratory. A portion of the samples should also be placed in a sterile tube containing PRAS medium if an anaerobic culture is required.

NOTE: Syringes with the needle attached should not be accepted due to the sharps and biohazard risk to staff. Syringes capped with a Leur-Lok are also not acceptable because the specimen may leak during transport and the samples may also be contaminated during handling.

d. A biopsy sample of the abscess capsule or tissue may also be collected and sent for culture during surgical incision and drainage procedures.

3. Drainages

See procedure 3.5 and Table 3.5–2 for drainages that are acceptable for culture. Drainage tube devices should not be cultured. Abdominal, chest tube, and biliary t-tube drainages are the most commonly submitted drainages. Drainage fluids for culture should never be collected from the bag due to organism overgrowth in fluid samples that are not freshly collected and the concerns about contamination by skin and other normal microbiota in the area being drained. Drainage fluid should be freshly collected by direct aspiration of fluid from the area being drained or by aspiration of fresh fluid in the drainage tube after decontamination of the surface of the device. Cultures of surgical drainage from clean surgical procedures are not indicated if there are no signs of infection (6).

a. Disinfect the collection tubing, and aseptically aspirate fresh fluid from the tubing.

b. Submit the drainage fluid in a sterile, leakproof container.

c. Do not inoculate blood culture bottles with drainage fluids, because a Gram stain and culture correlation is needed to determine the clinical significance of drainage fluid isolates.

NOTE: Drainage tubes and tubing should be rejected for culture. Physicians may be educated about the proper collection of drainage fluid samples if a comment is included on the report such as, “Drainage tubes of this type are not acceptable for culture because of the high rate of contamination by normal skin flora. Drainage fluid is acceptable for culture if a fresh sample is aseptically collected by aspiration into a sterile container after disinfecting the collection tubing.”

4. Tissues and biopsy samples

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

b. If anaerobic culture is required, a separate piece of tissue should be submitted in a sterile tube containing PRAS media.
In the setting of orthopedic prosthetic joint infections, multiple tissue samples (3, 4, 5, 6) are recommended. The entire prosthetic joint may also be submitted for sonication and culture of the sonicated material. See Appendix 3.13.1–1 for sonication procedure.

5. FNA
   a. Insert the needle into the tissue, using various directions, if possible.
   b. If the volume of aspirate is large, remove the needle and submit with Luer-Lok on the syringe.
   c. If the volume is small, aspirate the specimen into the sterile locking microcentrifuge tube containing broth by drawing up and down to release the specimen from the syringe.
   ☑ NOTE: Always use a safety device on the needle. Do not submit needle to the laboratory.

C. Label specimen and requisition
   1. List demographic information on the patient.
   2. Describe the type of specimen (deep tissue, superficial tissue, decubitus, catheter site, boil, abscess, cellulitis, aspirate, pus, drainage, surgical incision site, etc.)
   3. State anatomic location (arm, leg, etc.)
   4. Record collection time and date.
   5. List diagnosis or ICD9 code, including cause and clinical signs of infection.
   6. List antimicrobial therapy prior to specimen collection.
   7. Choose tests requested, including anaerobic culture, if appropriate.
   ☑ NOTE: To avoid the overuse of full fungal cultures that require incubation periods of greater than 1 week, the laboratory can offer a fungal culture with a shorter (2- to 4-day) incubation period for yeast. Such cultures are useful and cost-effective for the diagnosis of nosocomial, foreign-body, and postoperative infections, where the likely pathogen is either bacteria, Candida species or Aspergillus species. Candida or Aspergillus species will grow on routine bacterial culture media within 1 week; however, a selective fungal medium may be indicated for cultures expected to contain mixed microbiota. Full fungal cultures for filamentous fungi and slower growing yeasts should be reserved for diagnosis of chronic infections or those in immunocompromised hosts, particularly those caused by dematiaceous and biphasic molds, and should be performed only from tissue, aspirate, or fluid specimens (not submitted on swabs).

D. Deliver aspirates and tissues to the laboratory within 30 min for best recovery.
   1. Keep tissues moist to preserve organism viability.
   2. Do not refrigerate or incubate before or during transport. If there is a delay, keep sample at room temperature, because at lower temperature there is likely to be more dissolved oxygen, which could be detrimental to anaerobes.

E. Rejection criteria
   1. Do not accept specimens for microbiological analysis in container with formalin.
   2. If numerous squamous epithelial cells are present on the Gram stain, especially from swab specimens, request a recollection if there is evidence of infection.
   3. Discourage submission of specimens to determine if an infection is present.
   4. Since commercial swab transport systems have been documented to preserve most pathogens for up to 24 h, swabs are generally not STAT transported either in the hospital or in the ambulatory setting.
   5. For multiple requests (acid-fast bacilli, fungal, bacterial, and viral) but little specimen, contact the physician to determine which assays are most important and reject the others as “Quantity not sufficient.”
### III. MATERIALS

#### A. Media

1. BAP

2. CHOC for surgical tissues, closed aspirates, biopsy samples (including respiratory sources), and FNA specimens or specimens from genital sites to culture for *Neisseria* or *Haemophilus* spp.

3. MAC or EMB, except for clean surgical specimens (e.g., orthopedic cultures). When in doubt, examine Gram stain to determine the likelihood of a mixed culture.

4. Phenylethyl alcohol agar (PEA) or Columbia colistin-nalidixic acid agar (CNA), if source (e.g., gastrointestinal) or Gram stain indicates that the culture contains Gram-negative rods, which may inhibit Gram-positive organisms.

5. Thayer-Martin or similar selective media for genital specimens or for other specimens if *Neisseria gonorrhoeae* is suspected.

6. Anaerobic culture media, if appropriate for site of collection and transport conditions. See section 4.

7. Special media for recovery of certain fastidious or unusual organisms, if requested. Refer to the table of contents of this section for procedures listed by specific microorganism names.

8. Tissue broth cultures

   Broth culture of deep tissues may increase the detection of small numbers of organisms. Biopsy samples from deep tissues (lung, brain, liver, bone, etc.) and other FNA samples may be inoculated to enrichment broth. Common broth media include the following.

   a. Anaerobic BHI or TSB with 0.1% agar with or without yeast extract

   b. Fastidious anaerobic broth (Quebec Laboratories, Inc., Montreal, Quebec, Canada)

   c. Although THIO is an excellent medium for anaerobe recovery, it is the least suitable for recovery of low numbers of aerobic organisms, including yeasts (7).

   **NOTE:** Broth cultures inoculated as an adjunct to direct plating of wounds and fluid samples have been shown to seldom yield results that would alter patient management and could be omitted for most specimens without compromising patient care (8). Broth cultures should also not be done on swabs obtained from superficial or deep wound sites (9).

#### B. Tissue-homogenizing apparatus

1. Scalpels and petri dishes (Fig. 3.13.1–1)

2. Mortars and pestles. Use only in an anaerobic chamber if anaerobes are suspected, as these devices aerate the specimen (Fig. 3.13.1–2).

3. Automated pummeling instrument (stomacher [Tekmar Co., Cincinnati, OH]; MiniMix [Interscience Laboratories, Inc., Hingham, MA]) (Fig. 3.13.1–3)

4. Commercially available disposable plastic grinding devices (BD Diagnostic Systems; Sage Products Inc., Crystal Lake, IL). These are safer than glass handheld grinders (Fig. 3.13.1–4)

#### C. Gram stain reagents

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**Figure 3.13.1–1** Illustration of sterile-scalpel method of homogenization of tissue.
IV. QUALITY CONTROL

A. Verify that media meet expiration date and QC parameters per CLSI document M22-A3 (10). See procedures 14.2 and 3.3.1 for further procedures, especially for in-house-prepared media.

B. QC each lot of CHOC (procedure 3.3.1) and N. gonorrhoeae selective agar (procedure 3.9.3) using appropriate microorganisms listed in these procedures.

C. Perform other user QC per CLSI document M22-A3 and section 14 of this handbook.
**V. PROCEDURE**

**Observe standard precautions.**

*It is imperative that these cultures be handled in a biological safety cabinet.*

A. For safety reasons, as with all microbiological specimens, and to protect the specimen from environmental contamination, place specimen and media in a biological safety cabinet or anaerobic chamber to perform specimen preparation and inoculations.

B. Inoculation

1. **Tissues**
   - a. Select a portion of the tissue biopsy sample for culture that is bordering and within the area of infection (i.e., necrotic tissue is usually at the center of infected tissue areas).
   - b. Perform anaerobic culture first, preferably in an anaerobic chamber.
     - (1) If the tissue is large enough to be safely handled, place the tissue in a petri dish or specimen cup and cut it in half with a knife. Cut a smaller piece from the half and immediately touch the cut surface to the inoculum area of the anaerobe plates, place the tissue into broth culture medium, and streak the anaerobe plates for isolation.
     - (2) Alternatively, and for smaller tissue specimens, grind or homogenize tissues in THIO or other reduced broth (Fig. 3.13.1–1 to 3.13.1–4).
     - (3) Incubate immediately.
   - c. If the tissue can be easily teased apart (e.g., lung, kidney, and brain tissue), cut a portion of the tissue into several pieces (use a sterile blade and stick or scissors) or gently tease it apart with sterile sticks (Fig. 3.13.1–1). (Save one piece of cut tissue without teasing for the smears.) Inoculate a piece of tissue onto each of the culture plates.
   - d. If the tissue is hard (e.g., bone and skin)
     - (1) A sterile scalpel may be used to chip off small bone pieces for culture from a larger specimen. This procedure, however, creates a safety issue for staff, and manipulation of the sample may cause contamination. Alternatively, viable tissue may be removed from the bone biopsy sample, and the tissue and bone may be plated to the same plates after the bone is sonicated (see Appendix 3.13.1–1).
     - (2) Alternatively, place some of the tissue in the grinding apparatus (Fig. 3.13.1–2 and 3.13.1–4) and grind with about 0.5 ml of fluid from broth culture medium.
     - (3) After homogenization, remove the homogenized specimen using a sterile pipette. Inoculate plates and place the rest into broth culture medium, if using.
V. PROCEDURE (continued)

NOTE: Always examine these specimens for the presence of any soft tissue. If you find any such material, carefully remove it with a sterile surgical scalpel. This tissue may be processed separately.

e. Use an automated pummeling instrument to grind tissues that cannot be easily teased, if available (Fig. 3.13.1–3).

(1) Place a portion of the specimen into a sterile bag along with a small amount of sterile broth. This aids the recovery of organisms from samples that remain intact after pummeling. The tissue and broth should both be cultured.

(2) Insert sample bag between door and paddles in blender, allowing 4 cm of bag to project above top of door.

(3) Pull handle forward to firmly close door, and switch machine on for 1 to 5 min.

(4) Switch machine off, hold bag, open door by lifting handle, and remove bag.

(5) Remove sample from bag by using sterile pipette, and inoculate media.

f. For Gram stain

(1) Touch preps of the tissue sample are made onto sterile glass slides before the culture is inoculated. If sterile slides are not used for the touch prep smears, then these should be made after the culture has been inoculated to avoid contamination.

(2) Make a fresh cut of tissue and prepare the smear by touching the tissue to the slide. If the tissue is hard and does not stick to the slide, place the tissue between the two slides and press the slides together. Then separate by drawing the slides against each other (see Fig. 3.2.1–1 and 3.2.1–2).

g. If the tissue is large enough, save an intact piece in the refrigerator for up to 7 days, or in the freezer for extended storage.

2. Aspirates and pus

a. Mix the specimen thoroughly. Place a drop of the specimen onto each piece of the medium.

b. If sufficient specimen is submitted, inoculate invasively collected aspirates to broth culture medium to make a 1:10 dilution. If the volume is small, omit broth culture.

c. Prepare smear for Gram stain by placing a drop of specimen on a slide and spreading it to make a thin preparation. If the aspirate fluid is clear, use the cytocentrifuge to concentrate the specimen for the smear.

d. If sufficient specimen is available, save a portion in the refrigerator for up to 7 days for further testing, if indicated.

3. Swabs

a. If an anaerobic culture is to be performed, inoculate anaerobic plates first.

b. Then place swab in 1 to 2 ml of broth and vortex.

c. Squeeze the swab against the side of the broth tube to express remaining fluid and then discard.

d. Inoculate aerobic plates and prepare smear for Gram stain as described for aspirates and pus.

e. Alternatively, the swab can be used for direct specimen plating. Always inoculate media from the least inhibitory to the most inhibitory.

f. Save broth in the refrigerator for up to 7 days for further testing, if indicated.

NOTE: Do not culture swabs from superficial wounds or abscesses in broth medium.
V. PROCEDURE (continued)

C. Aerobic incubation conditions (see section 4 for anaerobic incubation conditions and workup of anaerobic culture)

1. Incubate BAP, CNA or PEA, and CHOC in humidified incubator at 35 to 37°C with 5% CO₂. Incubate for a minimum of 48 h for open wound cultures and for 3 to 4 days for invasively collected specimens with no initial growth. Incubation may be extended to 7 to 14 days for invasive specimens (i.e., aspirated fluids and tissues) that remain culture negative after 3 to 4 days of aerobic incubation depending on the specimen source, organism of concern, or patient’s clinical history (see individual organisms and body sites in other procedures for guidance).

**NOTE:** Although *Propionibacterium acnes* is primarily anaerobic (see section 4), it can grow aerobically and thus require further incubation when infection with this organism is suspected (11).

2. Critical deep-wound, abscess, and tissue samples should have anaerobic cultures requested (see Table 3.13.1–3 and section 4) in order to recover all of the primary pathogen(s) causing infection in specific clinical conditions (brain abscesses, brain, lung, liver tissue, deep wounds, abscesses, etc.). The laboratory should also routinely do anaerobic cultures on these types of samples when the specimen Gram stain demonstrates purulence (i.e., PMNs) and one or more bacterial morphotypes suggestive of anaerobes.

3. Incubate MAC or EMB plates in ambient air at 35 to 37°C, unless it is inconvenient to keep them separate from the rest of the culture in 5% CO₂.

4. Incubate broth in ambient air at 35 to 37°C for 3 to 4 days. If possible, hold for 1 week at room temperature to ensure that some specimen is available if further testing is indicated.

D. Perform a Gram stain on all specimens and use in the evaluation of culture.

1. Refer to procedure 3.2.1 for details on smear preparation and staining.
2. Record the relative numbers of WBCs, epithelial cells, and bacterial and fungal morphotypes.

   a. If clinically important organisms are recognized or suspected (e.g., from a normally sterile site), based on the Gram stain interpretation, telephone or report results to the appropriate caregiver immediately. Report any bacteria seen in a surgically collected specimen from a normally sterile site. Some examples of significant bacteria include the following.

   (1) *Clostridium*-like Gram-positive rods seen on specimens from soft tissue infections or aspirates even in the absence of numerous intact PMNs

   **NOTE:** Clostridia and other anaerobes produce phospholipases and lipases, and some aerobes (e.g., *S. aureus*, group A *Streptococcus*, *Listeria*, and *Corynebacterium* spp.) produce phospholipases that can destroy host cells, so a reduced or absent number of PMNs may be found in the direct specimen Gram smear.

   (2) Numerous PMNs and Gram-positive cocci in clusters resembling *Staphylococcus* in an abscess or tissue

   (3) Bacteria seen from brain abscess specimens

   (4) Gram-positive cocci in chains suggestive of streptococci from endometrial tissue may be found in group A streptococcal puerperal sepsis.

   b. If multiple morphologies are seen on the smear and the culture was not inoculated onto selective agar, go back to the specimen and inoculate it to CNA or PEA and EMB or MAC.

E. Culture workup

1. Read plates and broth daily. Refer to procedure 3.3.2 for description of colony types, initial reading, testing, and reporting preliminary and final cultures. For identifications refer to Table 3.3.2–5 and procedures 3.18.1 and 3.18.2.
V. PROCEDURE (continued)

It is imperative that these cultures be handled in a biological safety cabinet.

2. For cultures of lymph nodes, work in a biological safety cabinet, since some pathogens found in these specimens are hazardous, e.g., *Francisella*, *Mycobacterium*, and *Brucella*.

3. Refer to Table 3.13.1–2 for the list of the most common pathogenic organisms associated with wound infections and Fig. 3.13.1–5 for algorithm for extent of workup of cultures. Follow Fig. 3.13.1–6 and identify any number of the organisms listed.

4. Generally identify up to three microorganisms listed in Table 3.13.1–2 if any of the following is true.
   a. PMNs were present on direct smear.
   b. The specimen was collected from a normally sterile site.
   c. The specimen was of good quality (e.g., no or few epithelial cells present).
   d. The organism was seen on the direct smear.

5. Perform only minimal testing to indicate the type of microbiota present for noninvasively collected specimens with any of the following.
   a. Moderate or numerous epithelial cells present on the smear
   b. No evidence of infection on the smear (no PMNs) and no clinical information accompanying the specimen to indicate an infection
   c. $\geq$3 organisms growing in the culture. See exceptions for specific organisms in Fig. 3.13.1–6, which are generally always reported.

   NOTE: Save all culture plates with growth for several days in case further work is requested by the physician. Seven days is usually sufficient, but if space is a problem, transfer isolates to culture tubes for storage or save for a shorter period.

6. Identify any number of microorganisms that only grow on CHOC, and not on BAP (*N. gonorrhoeae*, *Haemophilus*, and *Francisella*). Identify *Neisseria meningitidis*.

7. Identify *Streptococcus pyogenes* or *Streptococcus agalactiae*.

   NOTE: Notify the physician of the isolation of *S. pyogenes*, as it may represent a life-threatening case of necrotizing fasciitis.

8. *Staphylococcus*
   a. *S. aureus*
      (1) Perform AST from invasively collected specimens and from others, if the Gram stain indicates a good-quality specimen and an infectious process with this organism (e.g., PMNs with few or no squamous epithelial cells and staphylococci seen on specimen Gram stain).
      (2) If an infectious process is not apparent but it is the hospital policy to track nosocomial methicillin-resistant *S. aureus* (MRSA) infections, rule out MRSA on inpatient specimens, unless the patient has a prior positive culture with MRSA. Notify infection control practitioner if MRSA is present, per hospital policy.
   b. When coagulase-negative staphylococci are present, perform AST only if they are the only organisms isolated from invasively collected specimens, if they are associated with PMNs in the direct smear, or if they are isolated from multiple cultures. Report as normal cutaneous microbiota if found in mixed cultures in any amount from superficial wound specimens or if numerous epithelial cells are present in the specimen.

9. For *viridans group streptococci* or *enterococci*
   a. Identify at least to the genus level from surgically, invasively collected specimens where the organism is the single or predominant pathogen and the Gram stain indicates infection (the presence of PMNs).
Figure 3.13.1–5 Initial evaluation of positive wound cultures for organisms growing aerobically. Note: For lymph nodes, perform all work using a biological safety cabinet.
Figure 3.13.1–6 Algorithm to rapidly detect aerobic and facultatively aerobic microorganisms usually considered significant, even in low numbers or in mixed cultures. See procedures 3.18.1 and 3.18.2 for other tests needed to confirm suspected identifications.
V. **PROCEDURE (continued)**

b. Include in normal microbiota if found in mixed cultures and not predominant.

c. If determined to be a significant isolate or if indicated by infection control policies, perform a vancomycin screen on enterococci from inpatients and from transplant and oncology outpatients. Perform AST only if isolate is from normally sterile site (e.g., bone, brain) in pure or almost pure culture.

10. For **Gram-positive rods**, if specimen is from a normally sterile site or biopsy sample, rule out *Listeria, Erysipelothrix, Bacillus cereus, Bacillus anthracis, Arcanobacterium, Corynebacterium diphtheriae, Corynebacterium ulcerans, Nocardia,* and *Actinomyces*. Identify other Gram-positive rods if numerous and they are associated with PMNs in the direct smear or seen as predominant in smear or if isolated from multiple cultures. Otherwise include these in skin microbiota.

11. Include **yeasts** as part of normal microbiota unless predominant or numerous. Except for specimens from normally sterile sites, generally identify only *Candida albicans* to the species level.

12. For predominant or moderate to numerous amounts of **enteric Gram-negative rods**

a. If only one or two species are present or predominant and an indication of infection is seen on smear, identify and perform AST. For specimens from the abdominal cavity, the aerobic plates may contain only a few *Escherichia coli* organisms, but the smear appears to represent mixed morphologies. In such cases, do not set up AST on the *E. coli* until the results of the anaerobic culture can be evaluated. Potentially, the anaerobic microbiota may be the significant, predominant pathogen(s).

b. If enteric bacilli are few in amount or not predominant, or if >2 species are present with no predominant strain, report as “mixed GI [for gastrointestinal] microbiota.”

   (1) Rule out fecal pathogens (*Salmonella, Shigella, Campylobacter, and Yersinia* spp.) for specimens from abdominal abscesses.

   (2) Generally save a representative plate for up to 7 days in case further work is requested.

c. Identify and perform susceptibility tests on multiple morphologies of enteric Gram-negative rods only on special request after consultation with the laboratory director, designee, or physician.

NOTE: When cultures contain a variety of enteric rods, treatment must include a combination of antimicrobial agents that are known to eradicate normal intestinal microbiota. Examination of a culture with fecal contamination to detect and separate each species is futile and not helpful for overall treatment decisions.

13. For **Gram-negative rods** that are not of the *Enterobacteriaceae* family

a. Rule out organisms which are always considered pathogenic (e.g., *Brucella, Haemophilus, Pasteurella, Francisella*). Generally these organisms are recognized because they do not grow on MAC or EMB. Some of these organisms are common in bite wounds. Work in a biological safety cabinet, and see procedure 3.18.2 for identification flowcharts and tables. If *Francisella* or *Brucella* are suspected, all plates should be tape-sealed and further testing should be done in a BSL 2 cabinet, because they can cause laboratory-acquired infections.

   *Francisella* can be found in lymph node biopsy samples. It is a tiny coccobacillus that grows slowly and is catalase positive or weak and oxidase negative. It can ferment glucose, but it is negative for other biochemical tests. It is beta-lactamase positive. Refer to procedure 16.8 for further details.
V. PROCEDURE (continued)

Brucella can affect any organ system and thus may be present in abscess fluid, tissues, or aspirates of sterile sites. It is a tiny coccobacillus that grows slowly and is catalase and oxidase positive. It is urease positive. Refer to procedure 16.6 for further details.

b. Identify obvious P. aeruginosa (characteristic odor and beta-hemolytic colonies) and Stenotrophomonas maltophilia (yellow and oxidase negative). If in pure culture or significant amounts and the Gram stain suggests an infective process, perform AST.

c. Identify oxidase-positive, indole-positive organisms (Table 3.18.2–8) which are likely to be Aeromonas or Vibrio. Also examine for the pigmented Gram-negative rods Chromobacterium violaceum and Sphingobacterium.

d. Identify and perform AST on other Gram-negative rods (Pseudomonas species other than P. aeruginosa, Acinetobacter, and related non-glucose-fermenting rods) by following the algorithm in Fig. 3.13.1–5.

14. Refer to procedure 3.3.2 for details on handling of broth cultures.

F. Hold positive culture plates at room temperature or in the refrigerator for several days (generally 7 days) after the culture is completed for additional work if requested by the physician.

VI. INTERPRETATION

A. Continuous dialogue between the clinician or nurse and the microbiology laboratory should be encouraged for proper interpretation of results.

B. Reporting selected organisms in mixed cultures can lead to erroneous interpretation of the number and variety of infecting pathogens.

C. Performance of AST is not indicated in cases of mixed microbiota indicative of infection of the abdominal cavity with bowel contents. Treatment should include broad-spectrum coverage for normal intestinal microbiota.

D. Use of the Gram stain can improve the accuracy of evaluating the importance of each potential pathogen. Organisms present in the Gram stain of an appropriately collected specimen correlate with \( \geq 10^5 \) organisms per g of tissue (1, 6).

E. Clinical studies have demonstrated that the microbial load in an acute wound can predict delayed healing or infection. The more numerous the organisms, the more likely they are to be indicative of infection (1).

POSTANALYTICAL CONSIDERATIONS

VII. REPORTING RESULTS

A. Report Gram stain results as soon as possible, generally within 1 h for specimens from critical sites.

B. Report all negative cultures as “No growth in ___ days.”

C. If organisms are seen in the Gram stain and fail to grow in culture, indicate this in the final report stating the organisms failed to grow in the appropriate atmospheric conditions for that specimen (i.e., aerobic or anaerobic).

D. Report individually those organisms that are always considered pathogenic (Fig. 3.13.1–6) with enumeration, using a preliminary identification initially and the genus and species (if applicable) as the final identification, if applicable.

E. Due to their known virulence factors, indicate the presence of the following species.

1. Beta-hemolytic streptococci
2. S. aureus
3. P. aeruginosa
4. Clostridium perfringens

5. Report “Pigmented anaerobes,” Bacteroides spp., and “Mixed anaerobes” without further identification. (See section 4 for identification methods.)
### Table 3.13.1–4 Examples of mixed wound culture reporting

<table>
<thead>
<tr>
<th>Microbiological observation</th>
<th>Example 1</th>
<th>Example 2</th>
<th>Example 3</th>
<th>Example 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMNs in Gram stain</td>
<td>−</td>
<td>2+</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>Bacteria in Gram stain</td>
<td>3+ Gram-negative rods</td>
<td>3+ Gram-negative rods</td>
<td>3+ Gram-positive and -negative rods</td>
<td>No organisms seen</td>
</tr>
<tr>
<td>Bacteria in culture</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
<td>−</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>−</td>
<td>3+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1+ group A</td>
</tr>
<tr>
<td>Beta-hemolytic streptococci</td>
<td>3+ pure</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>3+ pure</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>(agents of gastroenteritis ruled out)</td>
<td>3+ pure</td>
<td>1+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>−</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td><em>Clostridium</em> spp.</td>
<td>1+</td>
<td>−</td>
<td>3+</td>
<td>−</td>
</tr>
<tr>
<td>Pigmented Gram-negative anaerobes</td>
<td>1+</td>
<td>−</td>
<td>3+</td>
<td>−</td>
</tr>
<tr>
<td>Nonpigmented Gram-negative anaerobes</td>
<td>1+</td>
<td>−</td>
<td>1+</td>
<td>−</td>
</tr>
<tr>
<td>Information provided on microbiology report</td>
<td>3+ *E. coli; 1+ anaerobic GI microbiota; 1+ <em>S. aureus</em></td>
<td>2+ <em>S. aureus</em> (AST); 3+ <em>P. aeruginosa</em> (AST); 1+ mixed skin and enteric microbiota</td>
<td>4+ mixed aerobic and anaerobic GI microbiota; 1+ <em>S. aureus</em></td>
<td>1+ *S. pyogenes; 1+ mixed skin and enteric microbiota</td>
</tr>
<tr>
<td>Notes</td>
<td>May rule out MRSA for infection control purposes. Save plates; may need to do AST on <em>E. coli</em> if diabetic, etc.</td>
<td>May rule out MRSA for infection control purposes.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Revised from reference 1.

Caution must be used in using the presence or absence of PMNs to report the presence of certain pathogens from mixed wound cultures. Clostridia and other anaerobes produce phospholipases and lipases, and some aerobes (e.g., *S. aureus*, group A Streptococcus, *Listeria*, and *Corynebacterium* spp.) produce phospholipases that can destroy host cells, so a reduced or absent number of PMNs may be found in the direct specimen Gram smear.

### VII. REPORTING RESULTS

(continued)

**F.** Report other pathogens, as indicated in Fig. 3.13.1–5, with either definitive or minimal identification, depending on quantitation, number of species present, and Gram stain results. (See Table 3.13.1–4 for examples of reports.)

**G.** Report AST on Gram-negative rods, enterococci, or *S. aureus*, using the flowchart in Fig. 3.13.1–5. In general, do not perform AST on microorganisms that are not predominant, are in mixed cultures, or are skin microbiota or if culture does not show evidence of an infectious process. Make exceptions to this general policy if requested to do so by physician caring for the patient or for infection control purposes.

**H.** For tissues associated with prosthetic material or prosthetic implants, use the flowchart in Fig. 3.13.1–5; however, skin flora can be pathogens in this setting, and AST should be set up when skin flora are present in multiple samples, there is evidence of an infectious process, or evidence from sonicated material.
REFERENCES


VIII. LIMITATIONS

A. The microbiologist plays a critical role in the treatment of wound infections because practitioners often consider the report from the laboratory as definitive proof of infection. Providing inappropriate identifications and susceptibility results can prompt unnecessary treatment.

B. The results of wound, abscess, and tissue cultures will only be as valuable as the quality of the specimen submitted, transport, and expedient processing.

C. The presence of PMNs is an indication of an inflammatory or infectious process, while the presence of epithelial cells indicates surface contamination of the specimen. Specimens containing numerous epithelial cells yield culture results of questionable accuracy in the diagnosis of the infectious process, and one can consider rejection of these specimens for culture.

D. If a patient is immunocompromised or has poor vascular supply, inflammatory cells may not be present in the specimen as a guide to the extent of workup of the culture.

E. Low levels of organisms or fastidious organisms that grow poorly on the direct plates may be missed in culture.

F. Antibiotics administered prior to sample collection may negatively affect the recovery of organisms associated with infection.

G. Many wound infections are polymicrobial, and the isolation of an organism in culture may or may not correlate with infection of the wound.

H. Unusual diagnoses and treatment considerations may alter the usual policies of the laboratory in workup of organisms and reporting AST.

I. The lack of isolation of a pathogen does not necessarily mean that the laboratory was unable to detect the agent. Other inflammatory diseases can have the same presentations as infectious diseases, including the presence of PMNs on the Gram stain.

J. For further details on reporting, refer to procedure 3.3.2.

VII. REPORTING RESULTS (continued)

I. When multiple morphologies are present, report with minimal identification.

Example: “Culture yields growth of >3 colony types of enteric Gram-negative bacilli. Consult microbiology laboratory if more definitive studies are clinically indicated.”

See Table 3.13.1–4 for other examples.

J. Additionally, if mixed microbiota are cultured with no predominant microorganism, report as GI, oronasal, skin, or genital microbiota. Use of selective media is helpful in evaluation of the polymicrobial nature of culture.

K. For further details on reporting, refer to procedure 3.3.2.
REFERENCES (continued)


SUPPLEMENTAL READING


APPENDIX 3.13.1–1

Method for Sonication of Applicable Prosthetic Material, Bone, and Tissue

I. PRINCIPLE

The culture of samples obtained by sonication of the explanted prosthetic joint is more sensitive than culture of periprosthetic tissue alone (1, 2). This procedure provides guidance for processing prosthetic joints, but it can also be adapted for use in processing any hard tissue or prosthetic material that cannot be easily ground or homogenized.

II. MATERIALS

A. Primary media

1. BAP
2. CHOC
3. Anaerobic media (i.e., BRUC), see section 4

B. Other supplies/equipment

1. Sterile Ringer’s Lactate Solution or broth culture media that support the viability of pathogens (see procedure 3.13.1.III.A.8)
2. Pipette
3. Sterile bent glass or plastic disposable sterile rods to spread inoculums
4. Ultrasonic cleaner (Aquasonic 750T; VWR Scientific, West Chester, PA; Fisher Scientific Ultrasonic cleaner FS110-D; Fisher Scientific, Pittsburg, PA)
5. Flat-platform vortexer
6. Conical 50-ml centrifuge tubes
7. Centrifuge


III. PROCEDURE

A. In the BSC, remove the lid from the sterile container with the explanted joint and add 400 ml of sterile Ringer’s lactate solution or selected broth. For smaller samples, place in a 10-ml sterile tube with screw top lid and cover sample with up to 10 ml of solution broth.

B. Replace the lid on the container and ensure that it is screwed on securely.

C. Vortex the container for 30 seconds using a flat-platform Vortex.

D. Sonicate for 5 minutes.

E. Vortex for 30 seconds.

F. Using aseptic technique, aliquot 50 ml of sonicated fluid into a conical 50-ml centrifuge tube for explanted joint specimens. For smaller samples, aliquot all sonicated fluid into centrifuge tube.

G. Centrifuge tube at 3000 relative centrifugal force for 15 minutes.

H. Remove 49.5 ml, all but 0.5 ml of supernatant (within 15 minutes to prevent resuspension of the sediment), and discard, leaving 0.5 ml remaining (100-fold concentration). This leaves the sediment in 0.5 ml of supernatant.

I. Place a drop of the sediment on a slide for Gram stain as described in procedure 3.2.1.

J. Drop 0.1 ml of sediment onto each blood agar, chocolate agar, and brucella agar.

**NOTE:** fungal media for yeast culture may also be inoculated. Refer to procedure for details.

K. Incubate BAP and chocolate agar in 5% CO₂ and Brucella agar in anaerobic conditions for 14 days.

L. Read plates daily.


References


3.13.2 Quantitative Cultures of Wound Tissues

PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE
Quantitative culturing is a patient management tool that can be used with a limited variety of specimen types. However, as indicated in the review by Bowler et al. (1), several publications have demonstrated a correlation between quantitative tissue biopsy cultures and the semiquantitative method of enumeration of organism growth (see Table 3.3.2–2) in a qualitative swab culture. Tissues from acute wounds, such as those from trauma and burn patients, and duodenal aspirates are the specimen types that may be used for quantitative microbiological analysis. This procedure describes collection and processing of tissue specimens and determination of bacterial counts. The presence of bacteria in tissue in significant amounts is one of a number of factors that have been associated with delayed healing and has also been correlated with infection. When tissue is not readily available, a swab sample may be a convenient substitute for a tissue biopsy sample, and, in a quantitative culture, it may similarly be an indicator of an infectious process. However, semiquantitative swab culture is generally sufficient for patient management (2).

Quantitative cultures for anaerobic bacteria are problematic and thus less meaningful. Anaerobic microorganisms tend to live in microbial synergy with other organisms in the culture and do not grow well when diluted.

Quantitation of bacteria in duodenal aspirates can predict defects in mobility of the intestines. See procedure 3.8.6 for details. For quantitative culture of specimens from bronchoscopy, refer to the respiratory procedure (see Appendix 3.11.2–1). This procedure contains information presented in procedure 3.13.2 by Mary K. York in the third edition of this handbook (3).

II. SPECIMEN COLLECTION, TRANSPORT, AND HANDLING

Observe standard precautions.

A. Specimen collection
1. Tissues
   a. Submit 2- by 1-cm or larger tissue sample collected after cleansing and/or surgical debridement to yield the most useful information. (Refer to procedure 3.13.1 for collection details). This will yield approximately 500 mg of tissue, depending on the density (4).
   b. Submit as soon as possible without transport medium, but keep moist in a sealed, sterile container.

2. Swab collection
   a. Refer to procedure 3.13.1 for collection.
   b. Use only alginate swabs for quantitative culture.

B. Rejection criteria
1. If insufficient specimen is received for quantitation, process the specimen for qualitative culture only.
2. Do not process dry swabs.
III. MATERIALS

A. Media
1. BAP or CHOC
2. EMB or MAC
3. THIO or saline for dilutions

B. Reagents
1. Stain reagents for Gram stain
2. 0.85% sterile NaCl
3. Sterile Ringer’s solution (available from suppliers of intravenous solutions) containing the following per liter
   a. 8.5 g of NaCl
   b. 0.3 g of KCl
   c. 0.33 g of CaCl₂

C. Other supplies
1. Loop method: use either platinum or sterile plastic disposable loops to deliver 0.001 ml (1 μl) or 0.01 ml (10 μl).
2. Pipettor method: sterile pipette tips and pipettor to deliver 10 or 1 μl
3. Sterile bent glass or plastic disposable sterile rods to spread inoculum (Excel Scientific, Victorville, CA; http://www.excelscientific.com)
4. Sterile pipettes
5. Analytical balance
6. Homogenizer (Omni mixer, Omni International, Kennesaw, GA; Polytron homogenizer, Kinetica Inc., Bohemia, NY) or automated pummeling instrument (stomacher; Tekmar Co., Cincinnati, OH)
7. CO₂ incubator at 35 to 37°C
8. Anaerobic atmosphere (optional)

IV. QUALITY CONTROL

A. Verify that media meet expiration date and QC parameters per CLSI document M22-A3 (5). See procedures 14.2 and 3.3.1 for further procedures, especially for in-house-prepared media.
B. Validate the method of quantitation using known cultures of various colony counts. Refer to Appendixes 3.12–3 and 3.12–4 for QC for loops and pipettors, respectively.

V. PROCEDURE

A. Specimen processing
1. Tissues
   a. Weigh the tube containing the tissue on an analytical balance.
   b. Remove the tissue by using aseptic technique, and place it in 5 ml of sterile 0.85% NaCl or THIO (6, 7, 8). Use THIO only if anaerobic culture is performed. This is a 1:5 dilution of tissue.
   c. Reweigh the now empty original specimen tube, and subtract to determine the weight of the tissue in grams or milligrams.
   d. Homogenize the tissue for 15 to 30 s. Keep the homogenate cool during processing. See procedure 3.13.1 for detailed tissue homogenization methods.
   e. Plate 0.1 ml of the original homogenate. Label plate “10⁻¹” for amount of dilution of original homogenate (which is a 1:5 dilution of the specimen).
   f. Make one to three serial 1:10 dilutions of the homogenate with 0.5-ml aliquots and 4.5 ml of sterile 0.85% NaCl per aliquot.
   g. Plate 0.1 ml of each dilution onto BAP or CHOC and EMB or MAC. Evenly distribute the inoculum with a sterile bent rod. Label plates “10⁻²,” “10⁻¹,” and “10⁻⁰.” Alternatively, for the 10⁻³ and 10⁻⁴ dilutions, inoculate 0.01 and 0.001 ml of the first dilution with 10- and 1-μl loops, respectively.
   h. Repeat inoculations onto anaerobic BAP, if desired.

Observe standard precautions.
V. PROCEDURE (continued)

i. Incubate aerobic plates at 35°C in 5% CO₂ for 18 to 24 h. Use an anaerobic atmosphere for anaerobic plates.

2. Swabs
   a. Place swab in 5 ml of Ringer’s solution. Vortex and remove remaining swab material. (Swab should dissolve.)
   b. Beginning with item V.A.1.e above, serially make 10-fold dilutions in 0.85% NaCl and process as for tissue.

B. Gram stains

1. Prepare Gram stain of tissue specimens by spreading a 0.01-ml aliquot of the homogenate on a 1- by 1-cm area of a glass slide (9).
2. Allow the slide to dry, fix with methanol, and Gram stain.
3. Examine 10 fields under a 100× oil immersion objective (see procedure 3.2.1 for reporting guidelines).
4. Organisms are visible in Gram stains when at least 10⁵ organisms per g of tissue are present in the specimen (9).

C. Examination of cultures: tissue and swabs

1. Determine the number of organisms per gram of tissue by counting the colonies on the plate that grew between 30 and 300 colonies.
   NOTE: Only use MAC or EMB if overgrowth on BAP or CHOC precludes accurate counting of Gram-negative bacilli.
2. Calculate the total number of organisms by using the colony count times the dilution factor (use 5 as the factor for the homogenate dilution and the dilutions labeled on the plates for subsequent dilutions) divided by the original weight of the tissue. There is no weight of the tissue for swab specimens. Use only dilution factors.
   Example: Tissue weighed 0.3 g.
   Count was 50 on plate labeled “10⁻³."
   
   \[
   \frac{50 \text{ CFU} \times 5 \text{ (homogenate dilution)}}{0.3 \text{ g}} \times 10^3 \text{ (plate dilution)} = 8.3 \times 10^5 \text{ CFU/g}
   \]

3. Report total count “per gram of tissue” or “per swab.”
4. Work up pathogenic organisms for identification and antimicrobial susceptibility testing (AST) according to procedure 3.13.1 when the count is >10⁵ organisms per g. Special consideration must be made case by case for counts of <10⁵ organisms per g, depending especially on the identity of the isolate(s) and the type of disease of the patient.

VI. REPORTING RESULTS

A. Report direct results of smear of tissues with enumeration of each morphotype.
B. Report wound cultures as total number of organisms per gram of tissue.
C. See procedure 3.13.1 for reporting predominant species and AST.

VII. INTERPRETATION

A. The probability that tissue from a traumatic wound or burn is infected or will fail to heal can be predicted from cultures showing >10⁵ organisms per g (1, 4, 6, 7).
B. Colony counts of approximately 10⁵ CFU/g of tissue were found to be equivalent to colony counts of 10⁵ bacteria/ml of specimen obtained on a moist alginate swab (10).
Aerobic Bacteriology

VIII. LIMITATIONS

A. These tests should be used only under certain circumstances and after consultation with the physician or clinical service.
B. Loops are not as accurate as pipettes to deliver the inoculum.
C. Spreading with a loop is not as accurate as spreading with a bent rod.

REFERENCES


SUPPLEMENTAL READING