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**PRINCIPLE:** The primary agents of skin and tissue infections are *Staphylococcus aureus, Pseudomonas 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 initially need to be 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 3.13.1–1). 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.

**SPECIMEN COLLECTION AND HANDLING:**

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.*
2. Cleanse skin or mucosal surfaces.
	1. 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.
	2. 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
6. Anaerobe transport vial for small tissues
7. Sterile cup for large tissues with nonbacteriostatic saline on a gauze pad to keep moist
8. 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.
9. 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.
10. Swabs (*ideally, submit two, one for Gram stain and one for culture*) in a swab transport system with Stuart’s or Amies medium to preserve specimen and to neutralize inhibitory effects of swabs.

**MEDIA SETUP:**

1. BAP
2. MAC
3. CNA
4. Choc- if source indicates (such as bite wounds)
5. Enriched Thio (tissue cultures only)
6. Gram Stain
7. Anaerobic Culture Media – if indicated

 \*Specialized media is generally not required for routine wound infection isolation. References should be consulted if unusual organisms are suspected or requested.

**CULTURE SETUP and Inoculation:**

**Tissues**

1. 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).
2. 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.
3. Alternatively, and for smaller tissue specimens, grind or homogenize tissues in THIO or other reduced broth . Incubate immediately.
4. If the tissue can be easily teased apart (e.g., lung, kidney, 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. Save one piece of cut tissue without teasing for the smears.) Inoculate a piece of tissue onto each of the culture plates.
5. If the tissue is hard (e.g., bone, skin) 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, place some of the tissue in the grinding apparatus and grind with about 0.5 ml of fluid from broth culture medium. After homogenization, remove the homogenized specimen using a sterile pipette. Inoculate plates and place the rest into broth culture medium.

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

**Aspirates and pus**

1. Mix the specimen thoroughly. Place a drop of the specimen onto each piece of the medium.
2. 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.
3. 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.
4. If sufficient specimen is available, save a portion in the refrigerator for up to 7 days for further testing, if indicated.

**Swabs**

1. If an anaerobic culture is to be performed, inoculate anaerobic plates first.
2. Then place swab in 1 to 2 ml of broth and vortex.
3. Squeeze the swab against the side of the broth tube to express remaining fluid and then discard.
4. Inoculate aerobic plates and prepare smear for Gram stain as described for aspirates and pus.
5. Alternatively, the swab can be used for direct specimen plating. Always inoculate media from the least inhibitory to the most inhibitory.
6. Save specimen 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.

Critical deep-wound, abscess, and tissue samples should have anaerobic cultures requested in order to recover all of the primary pathogen(s) causing infection in specific clinical conditions (e.g., 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.

***Perform a Gram stain on all specimens and use gram results in the evaluation of culture.***

**Culture workup**

**General Guidelines:**

1. Read plates and broth (tissues only) daily for 3 days.
2. For cultures of lymph nodes, work in a biological safety cabinet, since pathogens can be found in these specimens that are hazardous, e.g., *Francisella, Mycobacterium,* and *Brucella.*
3. Refer to **Table 3.13.1–1** 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 3.13.1–1** if any of the following is true.
	1. PMNs were present on direct smear.
	2. The specimen was collected from a normally sterile site.
	3. The specimen was of good quality (e.g., no or few epithelial cells present).
	4. 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.
	1. Moderate or numerous epithelial cells present on the smear
	2. No evidence of infection on the smear (no PMNs) and no clinical information accompanying the specimen to indicate an infection
	3. >=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 for 1 week in case further work is requested by the physician.
6. Hold positive culture plates at room temperature or in the refrigerator for 1 week after the culture is completed for additional work if requested by the physician.

**Organism Specific Guidelines:**

1. **Growth on Chocolate Only** - Identify any number of microorganisms that only grow on CHOC, and not on BAP (*N. gonorrhoeae, Haemophilus,* and *Francisella*). Identify *Neisseria meningitidis.*
2. ***Streptococcus pyogenes* or Streptococcus agalactiae** should always be identified*. NOTE:*Notify the physician of the isolation of *S. pyogenes,* as it may represent a life-threatening case of necrotizing fasciitis.
3. ***Staphylococcus***
	1. ***S. aureus*** *-*  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. **C*oagulase-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 flora if found in mixed cultures in any amount from superficial wound specimens or if numerous epithelial cells are present in the specimen.
4. **V*iridans group streptococci* or *enterococci***
	1. 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).
	2. Include in normal flora if found in mixed cultures and not predominant.
	3. If determined to be a significant isolate perform AST only if isolate is from normally sterile site (e.g., bone, brain) in pure or almost pure culture. Release “Drug of Choice is Penicillin” from clinically significant non-sterile site.
5. ***Gram-positive rods****,* if specimen is from a normally sterile site or biopsy sample, rule out *Listeria, Erysipelothrix, Bacillus cereus, Bacillus anthracis, Arcanobacterium, Corynebacterium ulcerans, Nocardia,* and *Actinomyces.* Identify other gram-positive rods if numerous or seen as predominant in smear. Otherwise include these in skin flora.
6. **Yeasts** - Include *yeasts* as part of normal flora unless predominant or numerous. Except for specimens from normally sterile sites, generally identify only *Candida albicans* to the species level.
7. ***Enteric gram-negative rods*** *– Predominant or* moderate to numerous amounts -
	1. 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).
	2. 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] flora”
		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.
		3. 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 which 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.
8. **Non-Enterobacteriaceae Gram Negative Rods** - For gram-negative rods that are not of the *Enterobacteriaceae* family
	1. 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. These organisms are not uncommon in dog and cat bite wounds. Work in a biological safety cabinet, and see procedure 3.18.2 for identification flowcharts and tables. Note: Francisella *can be found in lymph node biopsy samples and is extremely infectious. 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 bioterrorismprocedures for other details.*
	2. 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.
	3. 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.*
9. **Non-Fermenting Gram Negative Rods** Identify and perform AST on other gram-negative rods (*Pseudomonaceae, Acinetobacter,* and related non-glucose-fermenting rods).

**REPORTING RESULTS:**

1. Report Gram stain results as soon as possible, generally within 1 h for specimens from critical sites.
2. Report all negative cultures as “No growth in \_ days.” Negative cultures must be held at minimum for 3 days.
3. Report individually those organisms that are always considered pathogenic with enumeration, using a preliminary identification initially and the genus and species (if applicable) as the final identification, if applicable.
4. Due to their known virulence factors, indicate the presence of the following species under all conditions, even if mixed.
	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.)
5. Report other pathogens with either definitive or minimal identification, depending on quantitation, number of species present, and Gram stain results.
6. Report AST on gram-negative rods, enterococci, or *S. aureus,* using the flowchart in **Fig.** **3.13.1–5.** Generally 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.
7. When multiple morphologies are present, report with minimal identification**.** Example:“Culture yields growth of >=3 colony types of enteric gram-negative bacilli. Please notify the Microbiology laboratory if further workup is needed.”
8. Additionally, if mixed microbiota are cultured with no predominant microorganism, report as GI, oronasal, skin, or genital microbiota.

**INTERPRETATION:**

1. Continuous dialogue between the clinician or nurse and the microbiology laboratory should be encouraged for proper interpretation of results.
2. The results of wound cultures will only be as valuable as the quality of the specimen submitted, its transport, and expedient processing.
3. Reporting selected organisms in mixed cultures can lead to erroneous interpretation of the number and variety of infecting pathogens.
4. 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.
5. *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* >=*105 organisms per g of tissue*
6. 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.
7. Many wound infections are polymicrobic, and the isolation of an organism in culture may or may not correlate with infection of the wound.

**LIMITATIONS:**

1. 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.
2. 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.
3. 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.
4. Low levels of organisms or fastidious organisms that grow poorly on the direct plates may be missed in culture.
5. Unusual treatment considerations may alter the usual policies of the laboratory in reporting AST.
6. 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

**REFERENCES:**

1. Clinical Microbiology Procedures Handbook. 3.13.1 Wound Abscess and Soft Tissue Cultures. March 2007 Update.