Laboratory Diagnosis of Bone, Joint, Soft-Tissue, and Skin Infections

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The diagnosis of infections of bones, joints, skin, and soft-tissues requires the combined use of a number of laboratory and pathology tests. The diagnosis of most infections requires microbiological cultures, both for isolation and for identification of causative organisms, as well as for antimicrobial susceptibility testing. Chemical analysis of joint fluids and histopathologic examination of infected tissues are often necessary to distinguish infections from other causes of inflammation, as well as to provide information as to the type of infection before the results of cultures are available. At this time, the use of molecular amplification tests is of limited value in the diagnosis of these infections, their value primarily being as adjunct tests for the diagnosis of rare or unusual infections.

This review addresses the laboratory diagnosis of bone, joint, soft-tissue, and skin infections. We emphasize the diagnosis of common infectious diseases, particularly bacterial infections, using diagnostic methods that are readily available in most hospital laboratories.

GENERAL COMMENTS

Microbiological cultures. Infections in this group of tissues share several factors that are important for diagnosis and treatment. The most important factor is the need to collect specimens in such a way as to avoid contamination by skin flora or other normal flora from adjacent contaminated structures, such as the gastrointestinal, urogenital, or upper respiratory systems. Another important factor is the need to collect tissue or fluid specimens, rather than swab samples, for culture. Although swabs are inexpensive and easy to use, swab samples have the following disadvantages: (1) they are more likely than tissue or fluid specimens to be contaminated; (2) they contain and release insufficient volumes of specimen for culture [1]; (3) they inhibit the growth of certain pathogens [2]; (4) bacteria survive less well in swabs than in aspirated fluid or pus [3]; and (5) microorganisms may adhere to swabs and, therefore, not appear on Gram staining, giving a false-negative test result. For some infections, such as postoperative wound infections, use of fine-needle aspiration is superior to the use of wound swabs for obtaining specimens [4].

Histopathology. Histopathologic and cytopathologic examination, including Gram staining or examination of a frozen section of fresh tissue, can provide preliminary (and often definitive) diagnoses more rapidly than can culture, but they also may be necessary for assessing the clinical relevance of culture results, particularly when cultures yield bacteria that could be either pathogenic or a contaminant [5]. In this context, it is important to emphasize that a Gram staining of an impression smear is easier to perform and to interpret than tissue variations of Gram staining performed on paraffin-embedded tissue sections.

The tissue Gram stain is an insensitive test for detecting bacteria. Moreover, the processes of fixing and processing soft-tissue specimens expose microorganisms to formalin, ethyl alcohol, xylene, and paraffin embedding. In addition, bone specimens undergo chemical decalcification. Not surprisingly, fixation and processing of specimens affects the tinctorial characteristics of bacteria, which, as a result, may or may not exhibit typical Gram stain characteristics. In particular, gram-positive bacteria may lose the ability to retain crystal violet and appear to be either gram-variable or even gram-negative bacteria. Gram-negative bacteria may appear magenta and be misinter-
interpreted as gram-positive bacteria. It is also important to remember that, because of the different milieu in which they are growing, bacteria may not exhibit the same morphological characteristics in tissue that they exhibit when taken from culture media. For example, staphylococci may not form clusters, and streptococci may not form chains.

Histopathological evaluation of the margins of resected or excised specimens is important in the management of malignant diseases, but little or no information has been published regarding the utility of margins in the management of infectious diseases. Surgeons typically rely on the apparent viability of tissue at the time of surgery, but the gross examination of tissue may miss early involvement by infection, especially with respect to bone. Surgeons must balance the need to resect sufficient tissue to promote healing against the need to spare as much tissue as possible. As a result, the surgical margins may be close to the infected area. Infected tissue at or near the surgical margin may result in treatment failure, but additional research is needed to determine (1) the size of noninfected margins that predict outcomes, (2) whether involvement of resection margins by infection results in different outcomes based on the infecting microorganisms, (3) the predictive value of soft-tissue versus bone margins in cases of osteomyelitis, and (4) what type and duration of antimicrobial therapy can be used to overcome involvement of a margin by infection.

Nucleic acid amplification tests. There is little published information regarding the role of nucleic acid amplification–based methods for skin, bone, joint, and soft-tissue infections. There are reports of nucleic acid amplification–based detection of Mycobacterium tuberculosis in cases of extrapulmonary disease, but reported yields vary, and there are no published data regarding the use of nucleic acid amplification methods for the detection of most other pathogenic mycobacteria and fungi [6]. Until the results of controlled clinical trials designed to evaluate the usefulness of these tests become available, routine use of nucleic acid amplification is not recommended for the diagnosis of these infections.

**BONE INFECTIONS**

No single laboratory test can be used to make the diagnosis of osteomyelitis. Although the diagnosis usually is made on the basis of clinical and radiographic features, excisional bone biopsy may be necessary in cases in which clinical and radiographic features are not diagnostic. In all cases, it is important to isolate the infecting agent(s) for identification and antimicrobial susceptibility testing, which requires an adequate volume of tissue for the cultures that are indicated. In cases of temporally chronic osteomyelitis, culture of an infected bone specimen is necessary to establish the diagnosis [7]. Isolation of the infecting agent by blood culture may be possible if the patient has bacteremia or fungemia. Cultures of sinus tract specimens show a poor correlation with cultures of bone specimens and should not be performed [8]. Although serial measurements of serum C-reactive protein levels and erythrocyte sedimentation rates are commonly used to observe the response to therapy, the diagnostic value of these tests is limited because of their low specificity.

**JOINT INFECTIONS**

Analysis of joint fluid is useful in determining the cause of effusions, although there can be overlap in the clinical and laboratory findings in patients with joints that are infected and those with crystalline arthropathies. Joint fluid should be cultured, and both a cell count (with differential) and crystal analysis should be performed. Gram staining of the fluid may be helpful, but the sensitivity is low [9]. Aerobic bacterial cultures usually are sufficient; anaerobic bacteria are not common isolates from infected joints. Because most common pathogenic yeasts grow well on routine aerobic bacterial culture media, only rarely are fungal media necessary. Blood culture bottles should not be used to culture joint fluid; these bottles are designed to maximize recovery of bacteria and fungi from blood, and no manufacturer recommends using blood culture bottles for culturing joint fluid. Moreover, fluid inoculated into blood culture bottles is not available for Gram staining, cell count, or crystal analysis. Fluid aspirated from joints should be collected aseptically, transferred to a sterile container (or left in the syringe), and sent immediately to the laboratory for processing. Cultures for Neisseria gonorrhoeae should be either inoculated to selective media at the point of collection or transported immediately to the laboratory. Specimens to be submitted for mycobacterial culture do not require special handling.

**SOFT-TISSUE INFECTIONS**

Mycetomas. Mycetomas are characterized by the triad of tumefaction (swelling), draining sinus tracts, and the presence of sulfur granules (visible bacterial or fungal colonies). They may be caused by fungi (eumycotic mycetoma) or by aerobic actinomycetes (actinomycotic mycetoma) [10]. These infections are most common in tropical and subtropical countries and are uncommon in the United States. Of 366 isolates of aerobic actinomycetes submitted to the Centers for Disease Control and Prevention in the 1980s, the 2 most common agents were Nocardia asteroides and Actinomadura madurae [11]. Mycetomas are most common in the feet and hands, sites that are more likely than others to be injured [12, 13]. The diagnosis can usually be made clinically, but radiographs are required for assessment of the involvement of bone and joints [12]. Smears of granules yield rapid preliminary information as to whether
the infection is bacterial or fungal. If granules are present, they should be crushed and cultured for fungi and aerobic actinomycetes. If granules are not present, an excisional biopsy for culture and histopathologic examination may be necessary. Moreover, because cultures may be contaminated with bacteria or fungi from skin or sinus tracts, correlation with histologic sections may be necessary. Except for removing granules for analysis, material from sinus tracts should not be cultured, because this material is likely to grow mixed flora that does not represent the cause of infection.

**Necrotizing fasciitis.** Most cases of necrotizing fasciitis are caused by either *Streptococcus pyogenes* or *Clostridium* species. More recently, cases have been reported that are caused by methicillin-resistant *Staphylococcus aureus* [14]. Because infections caused by *S. pyogenes* are treated with a penicillin and clindamycin, whereas penicillins and other β-lactam agents have no role in the treatment of infections caused by methicillin-resistant *S. aureus*, microbiologic diagnosis of the causative agent is imperative to guide therapy. A Gram stain of either a tissue aspirate or of an impression smear from a biopsy should reveal gram-positive cocci in short to long chains without clusters, cases caused by clostridia show gram-positive cocci in short chains and clusters, and cases caused by *S. aureus*, microbiologic diagnosis of the causative agent is imperative to guide therapy. A Gram stain of either a tissue aspirate or of an impression smear from a biopsy specimen is often diagnostic: cases caused by *S. pyogenes* show gram-positive cocci in short to long chains without clusters, cases caused by methicillin-resistant *S. aureus* show typical features of gram-positive cocci in short chains and clusters, and cases caused by clostridia show gram-positive bacilli. It is worth remembering that necrotizing fasciitis can progress so rapidly that tissue necrosis occurs before an inflammatory infiltrate can develop and/or toxins produced by the bacteria can lyse inflammatory cells; thus, a Gram stain may show large numbers of bacteria with few or no neutrophils. The role of frozen section diagnosis is limited to those cases in which the clinical and radiographic findings are not diagnostic.

**Pyomyositis.** Infections involving skeletal muscle require prompt diagnosis and treatment. *S. aureus* causes >95% of cases in tropical areas and ~70% of cases in temperate areas. The remainder are caused by a large number of other bacteria and fungi. Skeletal muscle that is near the skin, such as in the limbs or superficial trunk, is readily accessible to either a fine-needle aspiration biopsy or to an open excisional biopsy. Access to deep skeletal muscles, such as the deep muscles of the limbs or the psoas muscles, requires use of a CT-guided fine-needle aspiration biopsy or an open excisional biopsy.

**Lymph nodes.** An excisional biopsy or fine-needle aspiration biopsy is indicated when lymphadenopathy is unexplained. Excisional biopsies are preferred, because fine-needle aspiration biopsies yield insufficient material for microbiologic evaluation and cannot be used to evaluate tissue architecture in cases of suspected lymphoma. Excised lymph nodes should be divided for histopathological examination, culture, and, if necessary, flow cytometric and cytogenetic analyses. Touch preparations are useful both in making preliminary diagnoses and in determining whether to submit the tissue for histopathological examination and/or culture. Frozen sections of lymph nodes are discouraged, both because of the risk of contaminating cryostats with infectious agents and because of the risk of a misdiagnosis (freezing artifact may confound the histopathological features of lymph nodes, and freezing is not recommended). Histopathological analysis can distinguish between lymphadenopathy that is benign, reactive, malignant (either primary or metastatic), or infectious. Histopathological interpretation allows for either rapid preliminary or, in many cases, definitive diagnosis.

A common pattern of infection in lymph nodes is that of granulomatous inflammation. The differential diagnosis of granulomatous lymphadenitis is broad (table 1), including bacterial, mycobacterial, and fungal infections, toxoplasmosis, sarcoidosis, Hodgkin lymphoma, and non-Hodgkin lymphoma. Clinicians requesting a lymph node biopsy should communicate directly (and in advance) with the surgeon, pathologist, and microbiologist to ensure proper specimen collection, handling, and processing. Far too often, lymph nodes are received already in formalin, precluding microbiological cultures, flow cytometry, and cytogenetics; in some cases, even histopathological examination is compromised.

Although there are no recent large series regarding the microbiological characteristics of infected lymph nodes, there is sufficient information from earlier studies to make recommendations. The most important cultures are fungal and mycobacterial, irrespective of patient age, immune status, and geographic location [15]. Cultures for *Bartonella* species are

<table>
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<th>Table 1. Causes of granulomatous lymphadenitis.</th>
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<td><strong>Class, species</strong></td>
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<tr>
<td><strong>Bacteria</strong></td>
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<tr>
<td><em>Bartonella</em> species</td>
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<td>Francisella tularensis</td>
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<td>Yersinia pestis</td>
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<td>Yersinia pseudotuberculosis</td>
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<td>Mycobacterium avium complex</td>
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<td>Mycobacterium scrofulaceum</td>
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<td>Mycobacterium tuberculosis</td>
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<td>Fungi</td>
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<td>Histoplasma capsulatum</td>
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<td>Sporothrix schenckii</td>
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<td>Coccidioides immitis</td>
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<td>Paracoccidioides brasiliensis</td>
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<td>Parasite</td>
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<td>Toxoplasma gondii</td>
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indicated in suspected cases of cat scratch disease; serological testing is an alternative diagnostic method.

SKIN INFECTIONS

**Cellulitis.** Cellulitis is most often caused by skin flora, particularly *S. aureus* and *S. pyogenes*, but may be caused by flora from nearby mucosal sites (as with Fournier gangrene) or even distant sites [16]. Diagnosis usually is made on the basis of clinical criteria alone. In most cases of cellulitis, the yield of a needle aspirate is low for either Gram staining or culture [17, 18]. When it is clinically important to learn the identity of the causative bacteria or for antimicrobial susceptibility testing, a punch biopsy of skin can be performed and the sample submitted for culture and, if clinically indicated, histopathological analysis. It is imperative that culture samples of skin or of tissues that are contiguous with skin are collected with good aseptic technique.

The role of blood cultures in patients with cellulitis is not clear. Several studies suggest that blood cultures are not necessary for most patients, only for patients with certain clinical findings. One report indicates that blood cultures are necessary only when there is severe infection or when the patient has certain predisposing factors [18]. Another report suggests that blood cultures are indicated for patients with cellulitis who have lymphedema, buccal or periorbital cellulitis, chills and high fever, or an infection associated with a fresh- or salt-water source of infection [19]. A recent report suggests that patients who have limb cellulitis are at increased risk of bacteremia when the infection is associated with the absence of previous antibiotic treatment, the presence of ≥2 comorbid factors, duration of illness <2 days, and proximal limb involvement [20].

**Ecthyma gangrenosum.** The diagnosis of ecthyma gangrenosum is based on the clinical history and findings of physical examination. A skin biopsy for histopathological analysis may be indicated, but the diagnosis is not made using microbiologic cultures of skin samples.

**Impetigo.** The diagnosis of impetigo is based on clinical history, physical examination findings, and, if necessary, culture and Gram staining. Most cases today are caused by *S. aureus*, with fewer cases caused by streptococci. There is no role for histopathological analysis in the diagnosis of impetigo.

**Special considerations: injection drug users.** Injection drug users are likely to develop skin and subcutaneous abscesses at injection sites. These abscesses may be caused either by the normal skin flora that occur at the site of infection or by oral flora on needles used for injection. Either an aspirate or a biopsy specimen of abscesses should be obtained for aerobic and anaerobic culture. Patients with serious infections may become bacteremic, in which case the causative agent may be isolated by blood culture.

**Viral infections.** If the clinical presentation of a viral skin infection is sufficiently characteristic (e.g., classical chicken pox), diagnostic tests are not necessary. In general, macular or maculopapular lesions require serological testing or culture of samples obtained from other body sites, such as urine or respiratory secretions. If the presentation is vesicular or bullous and the clinical diagnosis is not obvious, detection of virus in the lesion is the most straightforward approach [20]. Molecular detection of virus in cutaneous lesions using RT-PCR is available and represents the diagnostic approach of the future [21]; it is not yet in widespread use, because kits cleared by the US Food and Drug Administration are still in development. Although RT-PCR appears to be the most sensitive method for detection of herpes simplex virus, conventional viral culture is a sensitive means for detection of this virus. Culture is, however, much less efficient at isolation of varicella-zoster virus. (It should be noted that culture is the most specific diagnostic modality and is also essential should susceptibility testing of isolates be required.) Most isolates of herpes simplex virus can be recovered within 72 h after inoculation, and one-half are recovered in the first day. Varicella-zoster virus grows slowly and may require 7–10 days for isolation by cell cultures [20]. Recovery of both viruses can be accomplished more quickly by use of spin amplification (with shell vials) [22]. Inclusion of rhesus monkey kidney cells in the conventional culture battery will also enhance detection of varicella-zoster virus [23]. In addition, direct detection of virus by immunofluorescence or immunoperoxidase offers a rapid method that is more sensitive than culture [21]. Direct immunofluorescence is the equivalent of the light microscopic Tzanck test, but it provides greater sensitivity and distinguishes varicella-zoster virus from herpes simplex virus.

Rarely, other viruses, such as the poxviruses, may present as vesicular skin lesions. A history of immunization against smallpox or contact with a person who has recently been vaccinated should direct attention to the possibility of vaccinia virus infection. Vesicular genital lesions may be produced by vaccinia virus after contact with a cutaneous inoculation site [24]. If variola is a clinical consideration, specimens should be referred directly to a confirmatory laboratory within the Laboratory Response Network.

**SPECIAL CONSIDERATIONS: INFECTIONS RESULTING FROM PERIPHERAL VASCULAR DISEASE**

In its early stages, management of the infectious manifestations of peripheral vascular disease usually involves medical therapy guided by microbiologic cultures. In its later stages, management usually involves surgical debridement with or without amputation, in which case there may be little or no need for microbiologic cultures. In all cases, there is no justification for the collection of superficial specimens from ulcers: cultures of
ulcers typically grow mixed aerobic and anaerobic bacteria and cannot be used to guide therapy. Many microbiology laboratories report predominant species for mixed cultures, which are often bacteria such as *S. aureus*, *Pseudomonas aeruginosa*, or *Bacteroides fragilis*, because these bacteria are perceived to be of greater clinical importance than other bacteria. Even this practice has little scientific basis: there is no practicable way to determine whether a given species is an etiologic agent or represents colonizing superficial flora. Blood cultures may be indicated for patients in whom group B streptococcal infection is more likely. Taking culture samples of infected tissue prior to amputation or debridement is discouraged, because there is no reliable and consistent means of predicting whether a particular microorganism will emerge as the cause of any subsequent infection.

**CONCLUSIONS**

Although most bone, joint, skin, and soft-tissue infections are caused by a limited number of bacterial and fungal pathogens, there is often sufficient overlap in the clinical and radiographic manifestations that microbiologic cultures are necessary to guide medical and surgical therapy. Because many of these infections are caused by normal flora, specimens must be collected in such a way as to avoid contamination by flora from skin or the gastrointestinal, urogenital, or upper respiratory tracts. Tissue or aspirated fluid samples should be submitted for culture; there is no role for the use of swabs in collecting specimens for culture other than for viral cultures. The role of nucleic acid amplification assays is not clear, because there are no large-scale controlled clinical trials using commercial systems to establish the performance characteristics of these assays. These assays should not be used routinely as a substitute for culture, but they may have a role as an adjunct to the diagnosis of infections caused by mycobacteria, fungi, or fastidious bacteria.

**Acknowledgments**


**References**