ABSTRACT The immunocompromised host is a particularly vulnerable population in whom routine and unusual infections can easily and frequently occur. Prosthetic devices are commonly used in these patients and the infections associated with those devices present a number of challenges for both the microbiologist and the clinician. Biofilms play a major role in device-related infections, which may contribute to failed attempts to recover organisms from routine culture methods. Moreover, device-related microorganisms can be difficult to eradicate by antibiotic therapy alone. Changes in clinical practice and advances in laboratory diagnostics have provided significant improvements in the detection and accurate diagnosis of device-related infections. Disruption of the bacterial biofilm plays an essential role in recovering the causative agent in culture. Various culture and nucleic acid amplification techniques are more accurate to guide directed treatment regimens. This chapter reviews the performance characteristics of currently available diagnostic assays and summarizes published guidelines, where available, for addressing suspected infected prosthetic devices.

INTRODUCTION
Prosthetic devices are frequently used in the management of patients with underlying immune deficiencies. Examples of prosthetic devices include a variety of joints, ventricular-assist devices, intravenous (IV) catheters, reservoirs for drug delivery, and postcancer reconstructive implants. The spectrum of underlying immunocompromised conditions spans the gamut of innate and acquired deficiencies of antibodies and complement, iatrogenic cellular-immune suppression to maintain transplanted stem cells and solid organs, neutropenia complicating chemotherapy, and biologic manipulation of interleukins and tumor-necrosis factor in numerous disease states. Dysfunction can occur in any and all arms of the immune system with sometimes predictable, but often surprising, infecting organisms and presentations. Prosthetic devices are especially prone to infection, given that skin provides a platform for bacterial colonization and immune evasion after violation of the skin by insertion of the device. Prosthetic-device infections can pose many clinical challenges, in particular the diagnosis and treatment of associated infections.

Unique to the management of infected prosthetic devices are the concepts of the biofilm and the small-colony variant (SCV). Each contribute to the diagnostic and management challenges for affected patients and will be subsequently reviewed as general concepts, applied to the clinical approach for selected prosthetic-device infections. This chapter will address the spectrum of pathogens, the diagnostic laboratory techniques, and the management of infected indwelling-prosthetic devices as related to infections specific to the compromised host.

BIOFILMS AND SMALL-COLONY VARIANTS
Modern medical microbiology is grounded in laboratory techniques introduced in the late 19th century, in which bacteria grown in liquid derivatives of food extracts or in blood were identified as the cause of infectious
diseases. Bacteria under these conditions are free-living and autonomous without significant interaction; in modern nomenclature these bacteria are termed planktonic. Antimicrobial-susceptibility testing (AST) relies upon inhibition of bacterial growth in liquid media or on solid-agar surfaces, and in the presence of antibiotics. Antif infective therapies prescribed on the basis of these observations proved to be an extraordinary tool in the advancement of medicine, contributing to improved overall survival from previously, frequently fatal, infectious diseases such as pneumonia, meningitis, and bacteremia. However, the impressive successes of antibiotic therapy for common infections of the mid-20th century have not been reproduced in addressing the infections of prosthetic devices today.

The fundamental issue that drives treatment failure of prosthetic-device infections is the presence of bacteria that are attached to the device surface and embedded in a secreted matrix of adherent proteins, polysaccharides, nucleic acids, and lipids, which constitute a biofilm. It is estimated that nearly 80% of all infections, not just prosthetic devices, involve bacteria that exist in a biofilm (1). Common chronic infections, which are biofilm-based, include: dental caries, bronchiectasis, cystic fibrosis (CF), endocarditis, *Helicobacter pylori* gastritis, otitis media with effusion, and pulmonary tuberculosis (2). Moreover, healthcare-associated infections (HAI s), such as central-line associated septicemia, *Clostridium difficile*, catheter-associated urinary tract infection, prosthesis-related infection, surgical wound, and ventilator-associated pneumonia are all infections associated with biofilms (3, 4).

Bacteria adhere to surfaces, especially fibronectin-conditioned device surfaces, where colonies consisting of single or multiple species live within a self-generated extracellular matrix. This matrix serves as a protective environment and reduces exposure of the bacteria to host immune defenses and extreme environments, such as limited nutrients and oxygen, antibiotic pressure, varying pH, and other environmental stresses. Bacteria in this restricted environment are termed sessile, exist in a slow metabolic state, and exhibit altered growth characteristics and reduced susceptibility to antibiotic concentrations in far excess of those needed to inhibit planktonic cells (5). Other metabolically reduced phenotypes, often associated with biofilms and chronic infection, include the viable but nonculturable (VBNC) state, as well as the SCV. Both the VBNC and SCV can be transient conditions that are induced under conditions of stress, such as those mentioned above, and can return to normal when conditions improve (5, 6).

Moreover, the biofilm co-locates organisms in an organized “microcolony,” which communicate optimized survival strategies via differential gene regulation and phenotype expression (frequently as SCVs) (1, 7). The polymicrobial nature of biofilms, in which bacteria, fungi, parasites, and viruses can coexist, is underrecognized. Interactions between *Candida albicans* and staphylococci in polymicrobial biofilms provide mutual-survival advantages, in which synergistic mechanisms reduce the effectiveness of antimicrobials (8). In general, antimicrobial resistance in biofilms is due to a combination of poor antibiotic penetration into the biofilm, altered metabolic activity of the microorganism(s), and modified expression of the antimicrobial target, such that the microorganism(s) are rendered resistant (9).

In addition to presenting therapeutic obstacles, biofilms negatively impact the ability to culture microbes using standard tissue, fluid, and swab specimens from implanted devices, including orthopedic implants. Methods to disrupt biofilm formation and enhance bacterial identification have primarily involved mechanical disruption (i.e., vortexing, bead-beating, or sonication) of the infected material, followed by culture or nucleic-acid amplification (10). This approach has significantly improved the ability to identify the relevant microbiology of infected explanted-prosthetic joints and other devices (11). Multiple emerging methods that address the diagnostic approach to these difficult issues are discussed below (see Analytical Considerations: Biofilm Disruption).

A second concept critical to understanding the diagnostic complexities and management of prosthetic device infections is awareness of the SCV. SCVs are naturally occurring subpopulations of bacteria and appear due to random switching in gene expression (6). Due to alterations in electron transport or auxotrophy (e.g., hemin, menadione, or thymidine growth requirements for *Staphylococcus aureus*), SCVs are phenotypically unique colonies when grown on agar-based media (Fig. 1). In addition to smaller colony size, SCVs may demonstrate altered pigmentation, reduced hemolytic activity, and nonstandard biochemical reactions, collectively making identification and isolation of these organisms challenging (12). SCVs have been identified in many Gram-positive (e.g., staphylococci in orthopedic and device infections) and Gram-negative populations (e.g., *Pseudomonas aeruginosa* in CF patients), although most work in understanding the SCV has been done with *S. aureus* (6).

SCVs are selectively induced under stressful conditions, such as those that induce biofilm formation.
(e.g., antibiotic pressure), which could be important for prosthetic-device infection. SCVs demonstrate reduced metabolic activity and virulence, enhanced intracellular survival, and resistance to antibiotics. Moreover, patients with chronic infection (e.g., CF, prosthetic-joint infection (PJI), long-term antibiotic therapy (especially trimethoprim/sulfamethoxazole) or skin and device-related infections (e.g., osteomyelitis) have increased risk for developing SCVs (6, 13). SCVs, when isolated from the CF lung, are associated with poor clinical outcomes (14); however, that is not uniformly the case with SCVs isolated from PJIs. When compared to normal-phenotype staphylococci, SCVs did not increase the rate of treatment failure. Rather, treatment failure increased in patients with S. aureus infection, regardless of SCV, compared to those with S. epidermidis (15). Other studies suggest that PJI due to SCVs should be managed differently due to their tendency to relapse, despite antibiotic treatment (16).

**SPECIFIC PROSTHETIC DEVICES**

**Central Venous Catheters**

The use of the central-venous catheter (CVC) is ubiquitous in the immunocompromised patient and long-term IV management is common practice. Examples of cath-
eters commonly utilized include: peripherally inserted central catheter (PICC), cuffed-implantable catheters of the Hickman or Broviac type, and totally implantable access ports (TIVAP). Each has its own particular and relative risk of infection (by convention described as infections per 1,000 device days), which is additionally related to the catheter’s insertion technique, duration of use (and number of accessions), and site of use (inpatients are at greater risk of infection than outpatients, especially for PICC lines) (17, 18). CVC-infection rates are highly variable, but for outpatients with cancer, generally <3 bloodstream infections (BSIs) per 1,000 device days are observed. TIVAP are associated with an infection rate of 0.2/1,000 device days, cuffed-implantable catheters up to 2.2/1,000 device days, and PICC-infection rates variably range from 0.4 for outpatients and up to 5/1,000 device days for inpatients (18–20).

The cause of catheter infection is either due to hub/lumen contamination, especially for tunneled or cuffed catheters, or due to contamination at the insertion site along the external catheter surface. Infections identified at 14 days or greater after line placement are generally related to hub/lumen contamination (21). Similarly, device infections occur either at the insertion site, the tunnel, or the device pocket and can be the cause of primary bacteremia. Symptoms of a vascular-device infection include fever, systemic inflammatory response syndrome (SIRS), tunnel or pocket inflammation, and purulence or erythema at the insertion site; however, the absence of pus, erythema, and tunnel tenderness at the insertion/exit site in no way excludes a diagnosis of catheter-related infection (21, 22).

The etiology of these infections is quite varied and dependent in part upon the indication for the device. Infection is dominated by S. aureus, coagulase-negative staphylococci (CoNS), Gram-negative bacilli, and Candida species; the latter is especially associated with total parenteral nutrition and organ transplantation (21). Despite the commonalities, the diversity of reported isolates is nearly endless and potential pathogens include aerobic, anaerobic, and fastidious bacteria, acid-fast bacilli (AFB), and fungi. CVC infections in mixed populations of neutropenic and nonneutropenic hematologic malignancy patients demonstrate a predominance of Gram-positive infections (23). More recently, nonneutropenic patients with infected cuffed catheters have shown a predominance of Gram-negative infections (24).

Diagnosis
While it should be simple to define an infected device, the reality is that definitions are cumbersome, at times arbitrary, and the clinical diagnosis is sometimes uncertain. Accurately identifying an infected device is important to provide optimal patient care. The 2009 Infectious Diseases Society of America (IDSA) Guidelines for Management of Intravascular Device-Related Infection provides several recommendations regarding diagnostic procedures to establish a catheter-related bloodstream infection (CRBSI) (21). These definitions rely heavily upon blood cultures from the central line itself, if the catheter is not removed. In a patient with bacteremia or fungemia, line infection should be suspected when an intravascular device has been placed and ≥1 positive blood-culture result is obtained from the peripheral vein, a clinical manifestation of infection (i.e., fever, chills, and/or hypotension), and no apparent source for bloodstream infection (with the exception of the catheter) is observed. In addition, one of the following conditions must be met: A) positive paired-blood cultures (set from line and peripheral) obtained quantitatively showing a ≥3 fold density of organisms in the central line compared with the peripheral draw; B) central-line culture turns positive ≥120 minutes before the peripheral culture (e.g., a 2-hour differential time-to-positivity (DTP), which is an indirect effect of bacterial density at the time of inoculation; or C) a positive peripheral-blood culture and the same organism isolated from a catheter-tip culture (i.e., >15 colony-forming units (CFU)/plate from a semi-quantitative culture from the removed catheter tip or >10^2 CFU from a quantitative sonicate culture of the catheter tip). The diagnostic performances of the different laboratory methods are discussed in greater detail in the laboratory methods section (see Central Venous Catheters: Laboratory Diagnostics).

Management
Long-term catheters should be removed from patients with CRBSI associated with any of the following conditions: severe sepsis, suppurative thrombophlebitis, endocarditis, bloodstream infection that continues despite >72 hours of antimicrobial therapy to which the infecting microbes are susceptible, or infections due to S. aureus, P. aeruginosa, fungi, or mycobacteria (21). For CRBSIs due to less-virulent microbes that are difficult to eradicate (e.g., Bacillus species, Micrococcus species, or Propionibacterium species), catheters should generally be removed after blood-culture contamination is excluded. Salvage of catheters can sometimes be attempted, especially when using combined systemic and antibiotic lock treatment for less virulent and potentially treatable organisms, such as CoNS (21). Expert advice is recommended in the management of an infected intra-
vascular catheter, irrespective of whether it is removed or salvage is attempted.

**Breast Prostheses**

The number of women diagnosed with breast cancer who undergo complete mastectomy as their treatment of choice is increasing (25, 26). Many women choose to have breast reconstruction surgery following mastectomy to rebuild the breast using a prosthetic implant. Breast reconstruction after mastectomy for malignancy is associated with higher risk of infection given more extensive tissue damage, prior breast surgery, radiation or chemotherapy treatment, and axillary lymph-node dissection (27, 28). Postmenopausal status, diabetes, steroid therapy, longer operative times, immediate implant after mastectomy, and repeated implant placement are additional potential risk factors (28). Implant or saline contamination, poor aseptic technique during surgery, nonsterile surgical environment, and hematogenous spread from another source are potential causes of implant infection. Other surgical-related risk factors include: seroma or hematoma, tissue ischemia, and drain placement (28). Penetration of the breast (e.g., trauma, nipple piercing) and other skin irritations are also risk factors (27, 28).

**Diagnosis**

Breast tissue is not sterile. In the majority of cases, cultures of the breast and nipple recover organisms recognized as normal skin flora, such as CoNS and Propionibacterium spp. Acute infection postreconstruction usually occurs within the first month after implantation, with saline implants showing clinical infection earlier than silicone implants (28). The responsible microorganisms are generally Gram-positive (e.g., S. aureus including MRSA, CoNS, streptococci, lactobacilli, Corynebacterium, and Propionibacterium). Notably, staphylococcal and streptococcal toxic-shock syndromes are reported as early complications of breast reconstruction (27). Late infection tends to occur months or years after implantation. Secondary bacteremia or hematogenous spread is a common cause of late-onset infection. Moreover, a variety of microorganisms, including S. aureus, S. epidermidis, Enterococcus avium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Pasteurella multocida, and Bacteroides fragilis can be associated with late infection (27).

One cause of early reconstruction infection is due to nontuberculous mycobacteria (NTM), specifically rapid-growing species, including Mycobacterium fortuitum/ chelonae and M. abscessus, which are well-adapted to biofilm formation (29, 30). These organisms are known environmental organisms and thought to contaminate the surgical site perioperatively. Mycobacterial breast infections have been more frequently diagnosed in the southern United States due to generally warmer climates, which sustain environmental reservoirs. The median incubation period for these infections is approximately 4 weeks postsurgery, and the presentation is relatively bland and nonspecific with breast swelling, erythema, and drainage (31). Of note, suggestive histology findings may not be appreciated from an immune-compromised individual. Diagnosis to the species level is important, and optimally, formal drug susceptibilities are obtained to guide treatment (29). A clinically infected breast, negative routine bacterial cultures, and continued infection despite empiric antimicrobial therapy may indicate the need to investigate a mycobacterial etiology.

Another possible complication following breast reconstruction is capsular contracture. As the name implies, a capsule forms around the breast implant, resulting in a firm or hard breast. The scar capsule can also contract, causing pain and an abnormal appearance of the breast, which in turn may require surgical repair. The rate of capsular contracture ranges from 1% to 33% and risk factors for developing capsular contracture include: infection, hematoma, host response, and preoperative irradiation (27, 32). Normal flora of the breast, which includes organisms recognized as skin microorganisms, such as CoNS and Propionibacterium, are associated with capsular contractures (27, 33). It is believed that the skin flora creates a biofilm on the prosthesis, which is thought to be associated with capsular contracture and infection.

**Management**

The management of infected breast prostheses usually requires removal of the prosthesis followed by anti-infective therapy. Conservative management with various combinations of implant preservation or exchange and antiinfective therapy has been successfully employed in a select group of patients; however, particular pathogens are associated with failure of conservative treatment, including MRSA, Gram-negatives, and Candida parapsilosis (34). Management of a contracture is complex, often requiring revision of the breast implant (35).

**Prosthetic Joints**

Primary (first replacement) knee and hip arthroplasties are common procedures that are complicated by infection (usually within the first year) rates of <2% in large
population studies (11). Shoulder replacements carry similar risk; however, elbow replacements are associated with infection rates of approximately 3%, possibly reflecting a predisposed population of rheumatoid patients. The immunocompromised host, including patients with malignancy, exogenous immunosuppression, and rheumatoid arthritis, demonstrates increased risk of infection (11). Data for rheumatoid patients in particular shows escalated risk of infection, with primary knee replacements showing approximately a 1.5-fold elevated risk compared with a normal cohort (36). Revision (prosthesis replacement) surgeries in the rheumatoid host, for both infectious and noninfectious causes, carry even greater risk, approaching 30% reinfection rates for previously infected joints (37).

Prior joint surgery of nearly any type is a risk factor for future PJI. Synovectomy is an example of a well-described surgical method of local immune suppression that was often performed prior to the routine use of disease-modifying antirheumatic drug (DMARD) treatment of patients with inflammatory arthritis. Limb lymphedema, venous insufficiency, and peripheral vascular disease are other well-known causes of joint immune compromise. The effect of radiation on the local bone and soft tissue is a major risk factor for PJI, possibly through alteration of the host response. Finally, the local effect of injected medications, such as intra-articular corticosteroid, is often overlooked. Corticosteroid injection in and around arthritic joints is a widely used procedure that is associated with local immune suppression. The clinician may have to inquire about prior corticosteroid injection, as the clinical relevance may not be immediately apparent. The risk of total hip and knee infection is likely increased when the surgery is performed in the months following intraarticular corticosteroid injection (38, 39). The local immune effects of other injected medications, such as hyaluronic-acid preparations, prior to joint replacement, are not known.

The microbiology of the infected prosthetic joint is dependent upon the timing of the infection following joint replacement. Early PJI is arbitrarily defined as occurring within 3 months of surgery and is characterized by more virulent organisms (S. aureus, aerobic Gram-negative organisms) and polymicrobial infections (11). In the normal host, culture-negative infections are estimated to occur in 7 to 15% of PJI, usually all in the setting of a late infection (>3 months postimplantation). Recent antibiotic exposure and fastidious organisms are major risk factors for culture-negative infections (11, 40–42).

The immunocompromised host is at risk for infections that are not commonly found in otherwise-healthy hosts and the medical literature is replete with case studies of uncommon microorganisms infecting prosthetic joints. Nevertheless, 50% to 60% of PJIs in the immunocompromised host are due to common microorganisms, such as S. aureus and CoNS, and their detection differs little from the immunocompetent host (11, 43, 44). Other causative Gram-positive microorganisms include streptococci, enterococci, and anaerobes such as P. acnes, Clostridium spp., and Finegoldia magna. Rare Gram-positive causes of PJI include Lactobacillus and Listeria in the immunocompromised host (45, 46). Gram-negative bacilli are documented in up to 45% of PJIs and are typically associated with early-onset PJI (11). Causative Gram-negative microorganisms include Escherichia coli, Pseudomonas aeruginosa, other non-E. coli Enterobacteriaceae, and anaerobes such as B. fragilis (47). There are rare cases documented in which Pasteurella and Salmonella have been recovered from immunocompromised hosts (48, 49). Fungal infections rarely occur (approximately 1% of all PJI); however, the majority of cases (80%) involve Candida spp. (11). Similarly, mycobacteria are seldom identified as the cause of PJI, with Mycobacterium avium most commonly isolated from the immunocompromised host (11, 50, 51).

**Diagnosis**

The clinical diagnosis of PJI, particularly of the hip and knee, is often straightforward. The host inflammatory response to planktonic bacterial infection is robust with clinically apparent pain, erythema, swelling, induration, and wound or sinus-tract drainage. However, in the setting of a biofilm-forming, chronic PJI, particularly in the immunocompromised host, the clinical signs of PJI are subtle and readily attributed to other host comorbidities (52). Joint pain or loosening may be the only complaint in an otherwise unremarkable-appearing joint. Moreover, the diagnostic accuracy of operative cultures is limited, especially when empiric antimicrobial therapy is administered before specimens are obtained for diagnostic purposes; up to 12% of clearly infected joints yield negative cultures (42, 53).

Diagnostic criteria and comparative microbiologic techniques of varying complexity have been published and will be summarized (see Prosthetic Joint Infection: Laboratory Diagnostics).

The definition of a PJI was recently revised by both the Workgroup of the Musculoskeletal Infection Society (MSIS) and the IDSA (54, 55). There are minor differ-
TABLE 1 Guidelines for prosthetic-joint infection (PJI) diagnosis

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<tr>
<td><strong>Definite PJI:</strong> Sinus tract communicating with joint OR growth of same organism in 2 or more periprosthetic tissue cultures</td>
<td><strong>Definite PJI:</strong> Sinus tract communicating with joint OR growth of same organism in 2 or more periprosthetic tissue cultures OR the presence of purulence surrounding the prosthesis without an alternative cause</td>
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<td><strong>Possible PJI:</strong> Three or more of the following Minor Criteriaa</td>
<td><strong>Possible PJI:</strong> Any of the following</td>
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<tr>
<td>• Erythrocyte sedimentation rate (ESR) &gt;30 mm/hr AND C-reactive protein (CRP) &gt;10 mg/l</td>
<td>• Growth of a virulent organism (e.g., <em>S. aureus</em>) from a single tissue biopsy or synovial-fluid sample</td>
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<tr>
<td>• Synovial fluid white blood count &gt;3,000 cells/μl or ++ change on leukocyte-esterase test strip</td>
<td>• Presence of acute inflammation on histopathologic examination of periprosthetic tissue at time of surgical debridement of prosthesis removal</td>
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<tr>
<td>• Synovial fluid PMN≥ &gt;80%</td>
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<tr>
<td>• &gt;5 PMN/HPF on histologic analysis of periprosthetic frozen section</td>
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<td>• A single positive culture</td>
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aThe minor criteria thresholds apply to joints >90 days post implantation.
bPMN, polymorphonuclear neutrophils.
-HPF, high-power field (microscopy).

ences between the definitions (Table 1). Note these definitions are not tested in the immunocompromised-patient population and are not to supersede clinical judgment.

Histology

Both IDSA and MSIS diagnostic PJI criteria include periprosthetic inflammation from either frozen or permanent sections. A threshold of 5 polymorphonuclear neutrophils (PMN) per high-power microscopic field (HPF) is generally recommended as a lower cutoff and this corresponds to culture-proven infection, although 10 PMN/HPF may be more specific. Using positive tissue cultures as the gold standard, the positive-likelihood ratio was 10.3 and 16.9 for 5 PMN/HPF and 10 PMN/HPF, respectively, in a recent meta-analysis reporting frozen-section results (56). Negative-likelihood ratios were 0.24 and 0.27 for 5 and 10 PMN/HPF, respectively. Summary analysis was that the positive histology predicted 75% culture-positive infection and a negative histology predicted a 5% probability of culture-positive infection. The analysis seems to be subject to significant sampling error and varying pathological interpretation. The effect of inflammatory arthritis or other immunocompromised states on the histological analysis of periprosthetic tissues in the setting of PJI is unknown, but probably significant. Moreover, low-virulence organisms, such as *Propionibacterium* spp., may not generate a robust tissue inflammatory response on histological analysis (57).

Surrogate markers

Because of the limited sensitivity of culture in chronic biofilm-forming PJI, diagnostic efforts have expanded to include detection of not only the offending organism, but also the host response as a proxy of infection. Both IDSA and MSIS consider combined elevation of the C-reactive protein (CRP) and erythrocyte-sedimentation rate (ESR) as suggestive of PJI. A normal combined ESR and CRP carries a very low likelihood of infection (58, 59). Various cutoff values have been proposed; the MSIS lists values of ESR of 30 mm/hr and CRP 10 mg/L (see above), while the IDSA lists no specific values. The individual sensitivity and specificity of elevated ESR has been reported to be 75% and 70%, respectively, while elevated CRP was 88% sensitive and 74% specific from a pooled analysis (various cutoffs in these studies) (58). Elevated serum interleukin-6 in this same study was found to provide the best diagnostic accuracy with 97% sensitivity and 91% specificity.

Improved detection of the host-inflammatory response occurs with direct assessment of the synovial fluid. Synovial white blood cell (WBC) values greater than 1,700 for the knee with percent neutrophils greater than 65% are suggestive of PJI in patients without inflammatory-joint disease (60). The optimal synovial WBC value likely varies slightly between anatomic-joint location, and the exact best “cut-off” value is debated (61). As the WBC exceeds 3,000, the accuracy of the test has been reported to be 99% in the knee (62). Special care must be used in the evaluation of synovial WBC count in the postoperative setting as far higher synovial WBC counts are found routinely even in the absence of infection. A postoperative synovial-fluid leukocyte count ≥27,800 cells/μl and a differential of 89% PMNs is predictive of total-knee arthroplasty infection in the first three months postoperatively (63). Similar values have been reported for the hip (64).
Few reports detail either inflammatory markers or synovial WBC analysis in the differentiation of infected from noninfected prosthetic joints in the setting of inflammatory arthritis. It is generally assumed that the interpretation of results would be confusing and uninterpretable. However, in patients with inflammatory arthritis and suspected PJI, the ESR, CRP, and synovial WBC counts performed equally at cutoffs of 30 to 32 mm/hr, 15 to 17 mg/l, and WBC of 3,444 to 3,450/μl, respectively, with essentially equal sensitivities and specificities (65).

There are several promising tests which have yet to be extensively studied in patient populations. Positive leukocyte-esterase reactivity of ++ in synovial fluid via colorimetric-strip test has been shown in a single study to be 81% sensitive and 100% specific for PJI with a negative-predictive value of 93% (61). Synovial-fluid CRP has also shown promise as a potential marker of infection (66). Recently, synovial alpha-defensin (Synovasure, CD Diagnostics) has been Food and Drug Administration (FDA)-approved for the detection of PJI. Alpha-defensin is a protein found in synovial fluid, produced as part of the nonspecific host-immune response that is proposed to be sensitive and specific for bacterial PJI, despite recent antibiotic exposure (67, 68). To the authors’ knowledge, neither synovial leukocyte esterase, synovial CRP, nor synovial alpha-defensin has been studied in the immunocompromised host.

Management

PJI surgical and medical management is complex with many areas of uncertainty beyond the scope of this discussion. Guidelines are available, which review general indications for debridement and retention versus explantation, antibiotic-impregnated spacer placement, and appropriate antibiotic-treatment regimens (52, 55). Unfortunately, there are no guidelines that describe application of these principles to the immunocompromised host, whose management must be individualized based upon unique aspects of the underlying disease, specific treatments, and net state of immunocompromised status. Fig. 2 is an adapted flow diagram that provides general guidance regarding management of the infected-joint prosthesis (55).

Debridement and retention describes a surgical procedure, in which the joint is opened and flushed, devitalized tissue removed, polyethylene components are exchanged, but implanted metal components remain. This procedure is then followed with antibiotic treatment (intravenous, oral, or combinations of both) over several months. In a two-stage procedure, an antibiotic-impregnated spacer is placed to maintain the soft-tissue dead space; antibiotics are administered for 6 weeks, followed by implantation of the prosthetic joint. Difficult-to-treat organisms, which are expected to require two-stage revision include: fungi, MRSA, enterococci, Gram-negative organisms, SCVs, and other drug-resistant microbes (52, 55, 69).

SCV and the prosthetic joint

SCV staphylococci are well-known causes of PJI, especially in persistent or relapsing infection (15). On the other hand, PJI due to Gram-negative SCVs is a less-frequent occurrence, but is described for E. coli (70). Although SCV Pseudomonas aeruginosa and Stenotro-

FIGURE 2 Management of the infected total-joint replacement. One-stage exchange criteria: applies to hip prosthesis only, susceptible to antibiotics with good penetration, adequate soft-tissue coverage, adequate residual bone requires no bone grafting, use antibiotic-impregnated bone cement for reimplantation. Two-stage exchange criteria: nonhip prosthesis OR poor soft-tissue coverage OR prior infected prosthetic joint OR difficult-to-treat organism. Modified with permission from reference (55).
*Pseudomonas maltophilia* are more commonly associated with CF biofilms (71), they do have the potential to cause PJI (52, 56, 72). The clinical presentation of SCV is that of a chronic low-grade infection that appears to be atypical for the isolated normal-phenotype organism (i.e., low-grade infection with *S. aureus*) (73). This scenario should serve as a clue to the clinician regarding the potential presence of SCVs in the bacterial population, with management implications as described below.

SCV isolation may impact the management of the patient. SCV PJI have been considered by some investigators “difficult-to-treat infections” with increased risk of treatment failure and, thus, deserve special approaches, which include antimicrobial therapy and a two-stage procedure that avoids the use of an antibiotic spacer following joint resection (74). Conversely, a more recent study showed no correlation between treatment failure for patients infected with either normal-phenotype staphylococci (32%) or the SCV phenotype (24%) when managed with a routine two-stage treatment (15). SCVs should be suspected in patients with PJI subjected to prior suppressive-antibiotic use, previous PJI, and chronic symptoms (>1 year).

**LABORATORY METHODS**

**Preanalytical Considerations**

**Stains**

The Gram stain is one of the most important diagnostic tools in the clinical microbiology laboratory. When appropriate, the Gram-stain result is the first piece of descriptive information that the laboratory provides to the clinician. The Gram stain’s limit of detection is approximately $10^4$ to $10^6$ organisms/ml of specimen (75). However, the utility of the Gram stain to identify device-related infection is limited due to difficulty in identifying indolent organisms that are often present in low quantities (below the limit of detection), as well as a diminished ability to detect bacteria in biofilms.

Fluorescent-staining methods provide greater sensitivity than nonfluorescent methods (e.g., the Gram stain) and have the potential to reveal the presence of microorganisms on culture-negative devices (76). Examples of fluorescent dyes that stain nucleic acid include acridine orange (AO) (Fig. 3), fluorescein isothiocyanate (FITC), and 4′,6-diamidino-2-phenyl indole (DAPI). While these methods will not differentiate between live or dead cells, the LIVE/DEAD BacLight (Molecular Probes) distinguishes live from dead bacteria based upon the selective penetration of the cell membrane by two fluorescent agents: SYTO 9, which stains all viable cells green and propidium iodide, which stains nonviable cells red. These methods will reveal the cell morphology (e.g., bacilli, cocci, clusters, chains), but will not determine the Gram-stain reaction; therefore, positive fluorescent results require further analysis to provide a more comprehensive diagnosis.

**FIGURE 3** Acridine orange. Smear stained with acridine-orange fluorescent dye. Microscopic observation at 1,000X reveals cocci in clusters, bacilli, and budding yeast. Photo courtesy of Jeffrey W. Prichard, D.O., Geisinger Health System.
Culture

Just as an understanding of infectious etiology is required for appropriate test selection, knowledge of proper specimen collection is essential for generating diagnostic results. In other words, high-quality specimens yield high-quality results. Many factors impact the utility of diagnostic microbiology. For example, the number of cultures, type of culture (aerobic versus anaerobic), process steps such as sonication, incubation period, and media (agar versus broth-based) all have the ability to influence the microbiology result(s). Moreover, a positive culture does not always indicate infection; it may indicate specimen contamination. As mentioned above, normal skin flora, such as CoNS, Corynebacterium, and Propionibacterium, can cause true prosthetic device-related infection, thus distinguishing between contamination and infection can be difficult. Therefore, it is important that the clinician understand the pre-analytical process, such as proper test selection (advantages and limitations), specimen collection (tissue and fluid are best; do not submit swabs), and specimen transport (conditions and stability limits), for optimal microorganism recovery. Likewise, it is essential that the clinical microbiologist implement best practices to improve the diagnostic yield of the analytical process.

Depending on the type of infection, increasing the number of specimens may add value to the interpretation of results, thus positively impacting patient care. Increasing the number of blood cultures increases the likelihood of detecting bacteremia in adults; 90% of infections are detected with 2 blood cultures and up to 4 may be needed in a 24-hour period to detect up to 99% of bacteremias (77). Similarly, when determining whether a prosthetic joint is infected, increasing the number of intraoperative cultures (minimum of 5) improves the diagnostic accuracy (contaminant versus pathogen) of the overall culture in 34% of cases (78). Furthermore, isolation of the same organism in ≥3 cultures has a sensitivity and specificity of 65% and 99.6%, respectively, which also serves as the cutoff for a definitive diagnosis of PJI (79).

Increasing the number/type of media used in culturing practices can also provide improvements in microorganism recovery. Media selection is vital to all culture-based methods, such that utilizing the wrong media could erroneously lead to a false-negative result. Common media include sheep blood, MacConkey, chocolate, and a variety of anaerobic agars. Additionally, cultures may or may not include a broth-based media, such as thioglycolate broth or a blood-culture bottle media. Blood-culture bottles, while not FDA-approved for non-blood specimens, demonstrate increased organism recovery (17%) compared with standard culture methods for sterile body fluids, such as synovial fluid (80). Overall, the number of media and type of media (e.g., selective and/or differential) appears to improve diagnostic utility; the same organism growing on multiple types of media is less likely to be considered a contaminant.

Depending on the site/source of infection and clinical presentation, anaerobic culture may provide more detailed information about the etiology of infection. Of the common etiologic agents of prosthetic-device infection, Propionibacterium is one of the most fastidious and, although P. acnes can cause several types of device-related infections, it has a strong predilection for the shoulder, as evidenced by higher incidence of shoulder infections (75% to 83%) than other surgical sites (hip, knee, ankle, elbow) (57, 81). If P. acnes is suspected, then it may be necessary to notify the microbiology laboratory as a means to ensure that the organism is properly cultured to maximize recovery. In the case of PJI, extended culture incubation in thioglycolate broth increases the recovery of P. acnes from tissue and fluid specimens (57). When compared to standard aerobic and anaerobic cultures versus extended aerobic and anaerobic, extending incubation of both cultures improved the diagnostic yield by nearly 30% for P. acnes PJI. However, extending the incubation period more than 14 days increases the likelihood of recovering contaminants (both P. acnes and others) (57, 82).

Analytical Considerations

Biofilm Disruption

Appreciation of the association between biofilm formation and prosthetic-device infection has stimulated the development of various methods to release biofilm-associated microorganisms from infected tissue and/or prosthetic devices. Common to these techniques is the ability to dislodge bacteria from a sessile biofilm and then employ routine culture or molecular methods. Moreover, these biofilm-disruption methods are easy to use, require limited laboratory equipment, and can be applied to many types of tissue and implants (83).

Vortexing, bead-beating, and sonication are effective mechanical methods for biofilm disruption and show improved microorganism recovery for the diagnosis of PJI. When in vitro biofilms were subjected to vortexing alone, culture sensitivity improved (P = 0.025), compared to culture without mechanical disruption (84). Beadmill processing, also known as bead beating, is another mechanical method in which glass beads are
utilized to homogenize the specimen. When subjected to rapid agitation, the beads shear the specimen by disrupting the associated biofilm. Although bead beating improved culture sensitivity (84%), it also showed increased contamination rate (8.7%) (83). Bead beating requires the addition of external fluid and glass beads to the specimen for processing, which may contribute to the increased contamination rate. Sonication utilizes ultrasound to aid in the disruption of the biofilm and thus releases sessile bacteria into a suspension, which can subsequently be cultured. Additionally, studies show that vortexing in combination with sonication further improves culture sensitivity and is commonly employed as it is a simple way to optimize organism recovery (84, 85). It should be noted that Gram-negative organisms, especially E. coli, are more sensitive to sonication than Gram-positive organisms. The duration of sonication should be limited to 7 minutes at room temperature, as longer exposure to the ultrasound and warmer temperatures inhibit organism recovery (86).

Processing prosthetic devices with sonication is becoming the gold standard for the diagnosis of biofilm-related infection and is quickly gaining favor for improving the diagnostic yield of other infectious processes (11, 87). There are several prosthetic devices for which sonication has been valuable, including fracture-fixation hardware (e.g., pins, screws, plates, rods), cardiac-rhythm devices, urinary tract infections, and cerebral implants (88). In most cases, sonication improves the sensitivity in diagnosing device-related infections with specific performance characteristics and interpretation guidelines reported below for the device-specific infections related to this chapter (see Specific Prosthetic Devices).

**SCV Recognition**

Isolation, identification, and susceptibility of SCVs present challenges to the clinical microbiologist. The SCV grows slowly *in vitro* and is usually present mixed among the normal phenotype, which can readily overgrow and hide the SCV. Moreover, laboratory identification can be delayed due to the reduced biochemical activity of SCVs (12). For example, SCV *S. aureus* can appear as pinpoint, nonpigmented, nonhemolytic, and catalase-negative. Therefore, it is imperative that the clinical microbiologist understands the issues, appreciates the differences, and can recognize the SCV phenotype to avoid misidentification and misinterpretation.

Because SCV grow poorly, they do not usually grow, let alone perform well, in automated systems, thus making the use of commercial-identification systems impossible. Luckily, comparative-growth studies can establish that an isolate is in fact a SCV, such that the isolate in question is cultured on different common media, some of which have the ability to reverse auxotrophy. In ambient air at 35°C, SCVs grow approximately 10-fold smaller than the normal phenotype on blood agar (BA) and grow better on chocolate and/or Brucella agar after 24 to 48 hours (14). Furthermore, the inoculation of selective and differential agar (e.g., chromogenic agar for the identification of *S. aureus*) and conventional, non-selective agar (e.g., BA), can rapidly improve the initial detection of SCV isolates of *S. aureus*; however, the utility of chromogenic agar to establish the presence of SCV *S. aureus* directly from clinical specimens is unknown (89).

Gram-negative SCVs present similar difficulties in identification and susceptibility testing (72). In the case of *E. coli*, SCVs from a sonicated infected prosthetic joint displayed altered biochemical results to key identification features, such as lacking β-galactosidase activity, being nonmotile, and the majority of SCV isolates demonstrating negative indole reactions (70). Unlike the growth studies established for *S. aureus* SCVs, the SCV *E. coli* grew optimally on BA, which was determined via comparative growth studies on BA, Brucella, chocolate, MacConkey, Mueller-Hinton, Mueller-Hinton with sheep blood, and Trypticase soy agars incubated aerobically and anaerobically, at 35°C, for 24 to 48 hours. Moreover, the SCVs reverted to the normal phenotype after repeated subculture (3 passages), which subsequently permitted standard biochemical identification using commercial products (e.g., Vitek 2 or API 20E) (70).

Because conventional methods are often negative or require extended incubation, the development of molecular methods, such as polymerase chain reaction (PCR), has provided alternative means to identify SCVs. Species-specific gene detection, broad-range PCR (e.g., 16S rRNA gene sequencing), or matrix-assisted light-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) are all methods that can improve diagnostic sensitivity and reduce turnaround times, but are more expensive alternatives (6, 15, 90).

Due to the auxotrophic requirements of SCVs, their impaired growth makes automated AST useless, as they are unable to support the growth of SCVs. Additionally, manual AST (disk diffusion and Etest) is also not suitable, as SCVs do not grow on the minimal media (Mueller-Hinton agar) routinely used. Therefore, there are no standardized susceptibility-testing methods for SCVs and extrapolation of susceptibility of the related normal population to the SCV must be reviewed with
caution. For example, studies demonstrate that antibiotic-resistant SCVs are able to revert to the normal phenotype thus complicating AST and treatment options (91).

For staphylococci, one of the most important antimicrobial-susceptibility determinations is methicillin resistance. Detection of the \textit{meca} gene by PCR is considered the gold-standard method to determine methicillin resistance for both \textit{S. aureus} and CoNS SCV (6, 90). Although PCR is rapid, sensitive, and specific, it may not be feasible due to cost and technical resources. Alternative methods are available that detect the \textit{meca} gene product, penicillin-binding protein 2a (PBP2a), that are also rapid, cost-effective, and easy to use. Unfortunately, the performance of PBP2a latex agglutination can be unreliable when performed on SCVs; however, application of a large inoculum (100–200 colonies) improved the accuracy of the assay and highly correlated with \textit{meca} gene detection by PCR (90). Studies are needed to evaluate the performance of the next generation of PBP2a assays, in which immunochromatography is employed, on SVC staphylococci (92). More important, discrepancy between genetic and phenotypic methods to detect methicillin resistance of CoNS suggests that non-\textit{meca} mechanisms of resistance exist (15).

\section*{Molecular Methods}

Because culture-based methods lack sensitivity and can take several days to become positive, culture-independent methods for the diagnosis of prosthetic-device infection are increasing. The most common molecular methods for the detection of device-related infections are PCR-based. Although PCR is not a new technology, its application in the clinical microbiology laboratory is just now being accepted as the gold standard for diagnosing many infectious diseases. PCR-based assays have revolutionized clinical microbiology, as the technology is more accurate and rapid, compared to traditional culture methods, which were previously revered as the gold standard. PCR sensitivity comes from its ability to detect and amplify DNA, whether the organism is dead or alive; however, this can be both an advantage and disadvantage. For example, prior antibiotic treatment typically results in negative culture, but can be positive by PCR, thus providing diagnostic utility (41, 93, 94). On the other hand, false-positive results can occur when contaminating DNA is present, which can complicate the diagnosis and therapy. Therefore, PCR results must be interpreted in the presence of other clinical data (e.g., culture, pathology, surrogate markers) for the diagnosis of PJI.

Moreover, PCR has evolved such that different applications can be employed depending on the information needed. Species-specific and broad-range PCR are the most commonly used methods, with PCR/MALDI-MS, multiplex PCR, and microarray-based PCR emerging as new technologies for diagnosing device-related infection.

\subsection*{Broad-range PCR}

Broad-range PCR targets the ribosomal ribonucleic acid (rRNA) gene of different classes of microorganisms, including bacteria and fungi; 16S rRNA and 28S rRNA, respectively. In clinical specimens, rRNA-gene amplification and sequencing are common methods to detect and identify microbial DNA. Broad-range PCR can provide great diagnostic utility, especially when cultures do not correlate with clinical presentation.

When used as an end-point (positive or negative) diagnostic tool, broad-range PCR can be useful, especially if ruling out infection. For example, 16S rRNA-gene amplification can detect all bacteria (aerobic, anaerobic, mycobacteria, as well as culture-negative organisms). Moreover, when the 16S rRNA-gene product is sequenced, it can provide useful information for organism identification. Typically, the diagnostic sensitivity of this assay improves when an organism is observed microscopically (e.g., Gram stain, fungal smear, histology). One major limitation is polymicrobial infections, which cannot be identified using this technology; however, next-generation sequencing may be a valid option in the future.

\subsection*{Species-specific PCR}

The development of species-specific primers allows individual organisms to be targeted and amplified via PCR, which can be useful when a specific etiology (e.g., \textit{S. aureus} or \textit{C. albicans}) or a group of organisms (e.g., staphylococci or \textit{Candida} species) is expected. Because the primers target a very precise organism sequence, species-specific PCR is more sensitive than broad-range PCR. Additionally, the “end-point” detection (negative or positive) of the PCR product promotes rapid results, typically within 1 to 2 hours, compared to broad-range PCR and DNA sequencing. Development of alternative amplification methods (e.g., isothermal) promise to improve result turnaround time, from hours to minutes. Unlike broad-range PCR, species-specific PCR can be directed towards organisms of clinical significance, which can improve the diagnostic utility. On the other hand, the organisms that can be detected are limited to a single species and may limit the assay’s usefulness. Multiplex PCR incorporates multiple species-specific PCRs in one assay, which improves the range of organisms and allows the detection of polymicrobial infections (93).
All in all, molecular methods for the direct detection of microorganisms show promise for accurate diagnosis of PJI. At this time, culture remains a necessary diagnostic tool, especially for the detection of unusual organisms and antimicrobial-susceptibility testing.

**Specific Prosthetic Devices**

**Central Venous Catheters:**

**Laboratory Diagnostics**

Establishing that the cause of BSI is catheter-related has significant implications on the management of the patient, as catheter removal is recommended. The laboratory plays a vital role in aiding the clinical assessment of catheter involvement in patients with CRBSI. Laboratory confirmation of CRBSI requires the recovery of the same organism from either paired blood cultures (catheter and peripherally drawn) or cultures of the catheter tip/segment and peripheral blood. Although several methods are used for the diagnosis of CRBSI, only a few are routinely practiced due to ease of use, cost, and diagnostic value. Catheter-sparing methods include: paired quantitative blood cultures, DTP, and the acridine-orange leukocyte cytospin (AOLC) assay. Diagnostic methods in which the removal of the catheter is required include semiquantitative and quantitative catheter cultures. For improved diagnostic yield, all methods should be paired with peripheral blood cultures.

DTP is the easiest method for the laboratory to perform and it can provide valuable information to determine the role of the catheter prior to catheter withdrawal. Time-to-positivity (TTP) is simply the time it takes for the blood culture to indicate organism growth (positive) and this information can easily be retrieved from the automated blood-culture instrument. When the TTP of a catheter draw and a peripheral draw are compared, the DTP can be calculated and utilized to diagnose CRBSI. A definitive diagnosis of CRBSI can be concluded if the catheter-drawn blood culture becomes positive with the same organisms ≥120 minutes before the peripheral-blood culture. The pooled sensitivity and specificity of DTP for diagnosing CRBSI is 89% and 87%, respectively, in short-term catheters, compared to long-term catheters, 90% and 72%, respectively [95].

Furthermore, the IDSA CRBSI guidelines are specifically applied to bacteremia with only few clinical studies addressing the diagnosis of candidemia. More recent reports confirm that a DTP of ≥120 minutes remains valid for *Candida* species except *C. glabrata*, which is the same DTP for bacteremia; whereas for *C. glabrata*, the recommended DTP cutoff is ≥6 hours [96]. TTP studies have reported a cutoff of ≤30 hours to be consistent with catheter-related candidemia, with a sensitivity of 100% and specificity of 51% [97]. When faced with candidemia and possible CRBSI, there is no current standard to guide clinical decisions and multiple data points in combination (number of positive cultures, TTP, and DTP) should be considered [98].

Catheters that are removed for suspected infection can be subjected to culture if the diagnosis of CRBSI is uncertain. The semiquantitative method has been a longstanding and accepted technique that is highly sensitive for identifying catheter colonization. This technique, also known as the roll-plate method, requires rolling the distal 5 cm of a removed catheter tip at least 4 times across a blood-agar plate. Cultures with >15 CFU of growth indicate catheter colonization, with a reported sensitivity of 85% when paired with a positive peripheral-blood culture [95]. Sonication cultures of removed catheter tips show similar sensitivity to the semiquantitative methods and are no more accurate than the semiquantitative roll-plate method for documenting catheter colonization. The procedure is technically more demanding, not standardized, and cannot be recommended preferentially over simple semiquantitative culture methods [99].

Despite the general reliance upon cultures to make a diagnosis of CRBSI, there are stain-based diagnostic criteria with reasonable performance characteristics that have been applied to patients with suspected CRBSI. In support of nonculture-based approaches, live-dead staining of *Staphylococcus epidermidis* venous-catheter-sonicate fluid has convincingly demonstrated the presence of VBNC in febrile hospitalized patients [100]. AOLC paired with Gram stains of blood aliquots drawn through a catheter can be used to diagnose catheter infections, provides a rapid (30-minute) turnaround time, and does not require catheter removal [101]. Having a threshold of >1,000 CFU/ml, AOLC is less sensitive than blood-culture methods, which have detection levels of <250 CFU/ml, but compares favorably to peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH), which has a reported lower detection limit of 10^2 to 10^3 CFU/ml blood [101]. The AOLC and PNA-FISH methods have a reported sensitivity of 96% and specificity of 92% in stem cell-transplant recipients, and when compared to DTP methods, a 69% sensitivity and 100% positive- and 88% negative-predictive value [102, 103].

While reasonably straightforward in concept, there are several caveats which should be considered in making the diagnosis of CRBSI. For example, the number of recommended peripheral-blood cultures needed to make
the diagnosis of DTP is not stated in the IDSA recommendations; however, one peripheral-blood culture has a 92% sensitivity in documenting a peripheral bacteremia when employed in DTP studies (104). In multiple-lumen catheters, the lumen used for either blood-product infusion or parenteral nutrition is most likely to cause the CRBSI and should be preferentially cultured when prioritizing paired-blood cultures (105).

Moreover, few studies have reported DTP performance in the febrile neutropenic patient. The majority of bacteremias in these patients (only 28%) do not meet the DTP definition for CRBSI with other sources, including gastrointestinal tract and mucositis (106). The most notable concern is the increased likelihood for a blood culture drawn from a central line to be contaminated. Catheter-drawn blood cultures are frequently either discouraged or regulated by institutional policy to reduce contamination risks (107). Operationally, it may be increasingly difficult to obtain central-line blood cultures, which will negatively impact the clinicians’ ability to accurately diagnose a CRBSI. Lastly, it is common for patients with suspected CRBSI to receive antimicrobials as a means to salvage the catheter; therefore, it is important to remember that administration of antimicrobial agents prior to culture (blood or catheter culture) decrease diagnostic sensitivity of all methods and catheter removal should not be delayed when CRBSI is suspected.

Breast prostheses: laboratory diagnostics

Standard microbiologic techniques include Gram stain, aerobic cultures, and anaerobic cultures (incubated 14 days to maximize the recovery of Propionibacterium spp.). Intraoperative swabs and tissue or fluid biopsies are the gold standard to diagnose the clinically infected breast prosthesis. In the case of a culture-negative infection, specimens should also be cultured for the recovery of mycobacteria (27).

Sonication of excised breast implants has contributed to a greater understanding of the microbiology involved in asymptomatic colonization and subclinical infection with Propionibacterium and CoNS, especially in the setting of capsular contraction. Early studies established that scrapings of the implant biofilm are superior specimens compared to the capsule tissue or intracapsular fluid, such that culture positivity from each was 47.9%, 30.2%, and 21.8%, respectively (108). Furthermore, because the periprosthetic tissue is often colonized with bacteria, no association between positive culture and infection or capsular contracture could be established. However, recent studies that utilized sonication to dissociate the bacterial biofilm from the implant surface also revealed the presence of normal skin flora (CoNS and Propionibacterium). Forty-one percent of excised breast implants were culture-positive in patients with moderate to severe capsular contracture (implant firmness determinable by touch (moderate) or sight (severe)) (109). Additionally, there was a correlation between positive cultures and advanced levels of capsular contracture, where 60% of severe, 39% of moderate, and 19% of mild contractures were culture positive, respectively ($P < 0.001$), suggesting that biofilms may play a role in the pathogenesis of capsular contracture (110). The hypothesis that bacterial colonization is associated with capsular contracture was further supported by the findings that the presence of bacteria on a breast implant correlated with culture positivity, in which ≥20 CFU/ml of skin organisms were recovered from sonicate fluid (111). Sonication in addition to multiple routine tissue cultures of breast implants and tissue expanders when removed for suspicion of infection or capsular contracture have the potential to improve diagnostic sensitivity and specificity.

Prosthetic joint infection: laboratory diagnostics

Neither IDSA nor MSIS specify which microbiologic techniques are recommended for the diagnosis of PJI, except to state that aerobic and anaerobic cultures should be obtained. Depending on the capability of the clinical laboratory, the microbiology methods may vary greatly. In this section we describe how culture type (synovial fluid, periprosthetic tissue, or prosthetic-joint sonicate) can play an important role in assay performance for the diagnosis of PJI.

Synovial fluid culture

Preoperative arthrocentesis and synovial-fluid analysis (including cultures) are the initial diagnostic tests performed for patients with suspected PJI. The number of synovial fluids that are culture-positive ranges from 9 to 29% (112). A meta-analysis of preoperative hip and knee aspiration cultures using standard laboratory-plate methods to diagnose PJI showed an overall sensitivity and specificity of 72% and 95%, respectively (113). Compared to total hip arthroplasty (THA), total knee arthroplasty (TKA) displayed increased sensitivity (78% versus 70%) and similar specificity (96% versus 94%). The use of blood-culture bottles and the corresponding automated system for diagnosing PJI from synovial fluid showed increased culture sensitivity (90% to 92%), compared to intraoperative swab cultures (68% to
76%) and tissue cultures (77% to 82%) (114). Inoculating synovial fluid (≥1 ml per BacT/Alert FAN bottle) into blood-culture bottles increased organism recovery to 100%, compared to 72% (P <0.001) of routine culture for infected fluids (80). In addition to improved organism detection, other blood-culture systems (0.5 to 3 ml per bottle BACTEC Peds PLUS/F bottle) also showed statistically fewer contaminants compared with conventional agar-plate methods, one versus 11 (P = 0.006) (112). Moreover, the use of blood-culture bottles (1 to 3 ml each) for culturing synovial fluid displayed 91% sensitivity for acute infections, compared to 79% for chronic infections, with 100% specificity for both (115).

Synovial fluid PCR. Although PCR may be more rapid and sensitive, there are no FDA-approved assays for the detection of microorganisms from direct specimens for the diagnosis of PJI. Therefore, little is known on the utility of molecular methods for the diagnosis of PJI from synovial fluid. The detection of bacterial DNA using an end-point (positive or negative) broad-range PCR (16S rRNA gene) was highly accurate in a small study (n=11) in which 91% of synovial fluids were positive from defined PJI, compared to 73% of culture (116). Because the percentage of culture-negative cases decreased with PCR, the use of broad-range end-point PCR may be warranted in cases in which culture is negative.

Tissue culture
Intraoperative tissue-culture techniques for the diagnosis of PJI are likewise variable, without standardized recommendations. Although optimal processing of tissue specimens remains uncertain, swab cultures are highly discouraged for the diagnosis of PJI as they are less sensitive (70%) than tissue culture (93%) (117). Several studies describe a variety of modifications to improve culture performance, which include the utilization of broth media and extended incubation. Similar to the synovial-fluid studies, broth-based media perform better than traditional culture plates for tissue culture (118). Another study showed that inoculating unprocessed tissue specimens immediately into broth during surgery can also increase culture sensitivity from 83% to 95%, compared to delayed processing in the microbiology laboratory (119). Inoculating homogenized tissue into anaerobic and aerobic blood-culture bottles held for 5 days showed optimal sensitivity (87%) and specificity (98%) compared with direct plating, cooked meat broth, and fastidious anaerobic broth (118). Homogenized tissue (processed via vortexing with glass beads) that was inoculated (0.5 to 1 ml of the homogenate) into blood-culture bottles (BD BACTEC aerobic and anaerobic bottles) showed improved culture performance and reduced TTP (118, 120). TTP was shorter using the blood-culture bottles, such that 95% of aerobic and 96% of anaerobic organisms were detected within 3 days and 5 days, respectively, suggesting that extended incubation is not necessary when utilizing an automated blood-culture detection system (120). Moreover, at day 3 the sensitivity and specificity was 82% and 99%, respectively.

Current guidelines recommend that intraoperative Gram stain no longer be used to diagnose PJI. The sensitivity and specificity of intraoperative Gram stain is 7% to 27% and 99% to 100% and has a negative-predictive value of 57% to 79% and positive-predictive value of 92% to 100%, respectively (121–123). Although the Gram stain has limited utility to rule out infection, it should not be discounted, as it can guide clinicians to select appropriate empiric therapy when positive (124).

Tissue PCR
Several recent studies show that broad-range 16S rRNA-gene PCR lacks sensitivity, ranging from 16% to 70%, compared to the tissue culture range of 69% to 89% for the diagnosis of PJI from tissue (125, 126). Furthermore, when compared to tissue PCR (16%), tissue culture (69%), synovial fluid (72%), and sonicate fluid (77%), combining the sonicate fluid with PCR (78%) was even more sensitive (126). Broad-range tissue PCR alone has unfavorable diagnostic sensitivity and is not recommended; however, when combined with microarray-based technology it can improve assay performance.

A novel microarray-based assay for the detection of microorganisms from direct specimens (Prove-it Bone and Joint assay by Mobidiag, Finland) shows promise for the diagnosis of PJI from tissue and bone. First, broad-range PCR occurs, followed by hybridization, and finally microarray analysis for a total assay time of 3.5 hours. The PCR detects a variety of Gram-positive, Gram-negative, aerobes, and anaerobes, for a total of 60 bacterial and 3 resistance-gene (mecA, vanA, and vanB) targets. In a blinded, prospective study, the PCR-microarray detected 82% of PJs compared to 74% by culture (127). Of note, the PCR-microarray exhibited a 26% false-positive PCR rate, for an overall accuracy of 79%, which was significantly better than culture methods. However, of the patients who received antimicrobial therapy prior to sample collection, the PCR-microarray detected 67% of positive cases, whereas culture only revealed 22% positivity rate. Although not
FDA-approved, this data suggests that PCR-microarray can provide increased assay sensitivity in cases where antimicrobial therapy is underway.

**Sonicate-fluid culture**

Sonication-fluid culture for the diagnosis of PJI has repeatedly proven to be more sensitive than routine tissue culture, especially when the patient has received antibiotics prior to specimen collection. The seminal prospective comparison by Trampuz et al. demonstrated that sonicate-fluid culture was more sensitive than tissue culture, 79% and 61%, respectively (P <0.001), with specific similarities (99%) (10). Sonicate-fluid culture significantly improved diagnostic sensitivity by 30% for patients who received antibiotics prior to surgery (P <0.001). Depending on the affected joint (hip, knee, shoulder, or elbow), PJI studies show a range of assay sensitivity for sonicate-fluid culture as 67 to 89%, compared to 55% to 61% for tissue culture (10, 81, 128). A meta-analysis of 12 published sonication studies (including knees, hips, shoulders, elbows, and ankles) reporting 1,637 total patients showed a pooled sensitivity of 80% and specificity of 95% (129). Additionally, ≥2 positive sonicate-fluid cultures significantly increased the specificity by 13% (P <0.001), which is similar to previous data regarding the optimal number of tissue cultures necessary to make an accurate diagnosis of PJI (94). It should be noted that sonicate-fluid cultures provide no additional benefit over tissue culture for early (<3 months after surgery) PJI; however, such techniques should be reserved for late infection (>24 months after surgery) or a failing implant, in which infection is not suspected (130).

The laboratory process involves receiving the removed prosthetic device (joint, surgical hardware, etc.) in a previously sterile, large, hard plastic (1 liter, wide-mouthed, polypropylene jar) container. Upon receipt, fluid (usually Ringer’s solution) is added to the container and the fluid functions to evenly distribute the ultrasound to the specimen. It is a common practice to add a large volume of fluid (400 ml of Ringer’s solution) so that the entire prosthesis is submerged in the liquid that is essential to the process. The container is then vortexed briefly (30 seconds), subjected to sonication (5 minutes at room temperature at 40 kHz), followed by an additional brief vortexing step. At this point, the sonicate fluid is ready for inoculation (unconcentrated), or it can be centrifuged (4,000 rpm for 5 minutes) to concentrate the specimen. The resulting sonicate fluid can then be plated to solid (broth is optional) media for aerobic and anaerobic culture.

Sonication improves the number of recovered organisms, thus sonicate-fluid cultures yield higher CFU/ml than tissue culture (Fig. 4) and increase pathogen detection by approximately 30% (131). Typically, sonicate-fluid cultures are quantified to establish PJI and the cutoff that defines a positive sonicate-fluid culture is dependent upon the sonication steps employed. For example, if the sonicate fluid is unconcentrated when inoculated, then a ≥5 CFU/plate (of the same organism) cutoff is recommended, as it shows optimal culture performance (10, 129). If the sonicate fluid is concentrated prior to inoculation, then a ≥20 CFU/plate cutoff is recommended since higher numbers of organism are recovered due to the concentration step (81, 129). One study compared the sensitivity of using a low cutoff (≥1 CFU/ml, any growth) versus a high cutoff (≥50 CFU/ml) and showed that vortexing and sonication cultures displayed similar sensitivities (approximately 70%) at the lower cutoff, but at a cost of reduced specificity (92%) (132). When using a ≥50 CFU/ml cutoff, vortexing was clearly inferior to sonication (sensitivity 60% vs 40%, P = 0.15), with similar specificity (99%) (132). The advantages of vortexing include simplicity of laboratory methods and also avoiding the potential lethal effects of sonication on bacteria.

Several variations to the culture protocol have been investigated with the goal of further optimizing sonicate-fluid culture sensitivity. The utility of prolonged incubation time (up to 14 days) of sonicate-fluid culture is unclear due to conflicting data (131, 133). In one study, all cultures, including P. acnes (n=1), were positive within the first 7 days of incubation (133). In contrast, 48% of sonicate-fluid cultures were positive on day 2, compared to 26% of tissue cultures (P = 0.002), suggesting that organisms are recovered in less time with sonication. Moreover, a 7-day incubation period was sufficient for aerobic organisms, but 14-day incubation was necessary for optimal anaerobic organism recovery (129, 131). Others suggest that prolonged incubation for suspected P. acnes could be selectively applied for shoulder/elbow-prosthesis sonication (81, 129).

The use of blood-culture bottles to increase the sensitivity of sonicate-fluid culture has also proved to be a beneficial addition. Recent studies demonstrate that the inoculation of sonicate fluid into blood-culture bottles improved organism recovery by increasing culture positivity and reducing TTP (134, 135). Moreover, sonicate fluid was significantly more sensitive (88%) than synovial fluid (64%, P = 0.009) inoculated into blood-culture bottles, with sensitivities of 98% and 87%, respectively (P = 0.032) (135).
Despite improved sensitivity afforded by the addition of sonication, cases of culture-negative PJI still remain. One reason for this could be initial misdiagnosis, aseptic failure rather than PJI. In conjunction with the clinical presentation, other laboratory findings should be taken into account, such as surrogate markers and histological findings. Another reason could be inappropriate culture conditions (e.g., fastidious organism, unsuitable culture medium, insufficient incubation time, VBNC, etc.) were utilized to recover the organism. Lastly, previous antimicrobial therapy negatively impacts culture sensitivity.

Of the possible reasons given for negative cultures, molecular methods can improve organism detection for all, except aseptic failure (10).

**Figure 4** Prosthetic-joint culture comparison. (A) sonicate fluid with *S. pyogenes* (>100 CFU/10 ml) and *S. aureus* (20–50 CFU/10 ml), (B) periprosthetic tissue #1 with moderate *S. pyogenes* and few *S. aureus*, (C) periprosthetic tissue #2 with moderate *S. pyogenes* and few *S. aureus*, (D) synovial-fluid culture growing *S. pyogenes* only, no *S. aureus*.

**Sonicate fluid PCR: reduces the number of culture-negative cases**

Although PCR is a powerful tool for the purpose of detecting the presence of bacterial DNA, it is not widely utilized in clinical practice for diagnosing PJI.
studies show that the sensitivity of PCR and culture of sonicate fluid are not statistically different, especially when broad-range PCR is employed (41, 94, 136). Because broad-range PCR is prone to false-positive results, it should only be employed when infection is suspected. If the patient lacks signs of infection, positive PCR results may indicate contamination rather than true infection. PCR can be effective if utilized as an add-on test when cultures are negative and a high clinical suspicion for PJI remains.

On the other hand, there are several studies in which commercially available multiplex-PCR assays show promising results for the purpose of diagnosing PJI. Multiplex PCR originally designed to identify microorganisms from positive blood cultures can be useful for accurate diagnosis of PJI (93, 137). The FilmArray assay, which is designed to extract, amplify, detect, and identify bacteria and yeast from direct specimens, displayed an overall sensitivity of 53%, which was inferior to culture (69%) (137). The SeptiFast assay, which requires a separate manual DNA extraction prior to PCR, showed high sensitivity and specificity, 96% and 100%, respectively (93). These data suggest that DNA extraction plays an important role in assay performance and that species-specific PCR may reduce sensitivity as the assay is limited to the genetic targets included in the panel. Further studies are warranted to better appreciate assay utility and patient impact.

**SUMMARY**

Prosthetic devices are essential for managing patients with chronic diseases, including those with associated immunocompromised status. These devices improve the quality of life and treatment options for patients at the cost of risk for infections, which can be associated with both significant morbidity and mortality. The principles of biofilm management are universal for these device infections, and generalizations can be made for many devices, which are not included in this chapter, despite the paucity of publications. It is clear that diagnostic testing will need to evolve beyond the standard current microbiologic procedures to include nucleic-acid detection, enhanced culture techniques, novel microbe imaging, and local immune response, and these diagnostic trials will need to be linked to translational studies showing the impact on patient care and outcomes. Finally, the individual patient will need to be better understood in terms of their net state of immunosuppression or immunocompromised status, and where possible, be managed with preventative measures to mitigate infection risk.

**REFERENCES**


Prosthetic Device Infections


