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TITLE:
A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiologya

ABSTRACT:
The critical nature of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician/advanced practice provider and the microbiologists who provide enormous value to the healthcare team. This document, developed by experts in laboratory and adult and pediatric clinical medicine, provides information on which tests are valuable and in which contexts, and on tests that add little or no value for diagnostic decisions. This document presents a system-based approach rather than specimen-based approach, and includes bloodstream and cardiovascular system infections, central nervous system infections, ocular infections, soft tissue infections of the head and neck, upper and lower respiratory infections, infections of the gastrointestinal tract, intra-abdominal infections, bone and joint infections, urinary tract infections, genital infections, and other skin and soft tissue infections; or into etiologic agent groups, including arthropod-borne infections, viral syndromes, and blood and tissue parasite infections. Each section contains introductory concepts, a summary of key points, and detailed tables that list suspected agents; the most reliable tests to order; the samples (and volumes) to collect in order of preference; specimen transport devices, procedures, times, and temperatures; and detailed notes on specific issues regarding the test methods, such as when tests are likely to require a specialized laboratory or have prolonged turnaround times. In addition, the pediatric needs of specimen management are also emphasized. There is intentional redundancy among the tables and sections, as many agents and assay choices overlap. The document is intended to serve as a guidance for physicians in choosing tests that will aid them to quickly and accurately diagnose infectious diseases in their patients.

INTRODUCTION ::: EXECUTIVE SUMMARY:
Unlike other areas of the diagnostic laboratory, clinical microbiology is a science of interpretive judgment that is becoming more complex, not less. Even with the advent of laboratory automation and the integration of genomics and proteomics in microbiology, interpretation of results still depends on the quality of the specimens received for analysis whether one is suspecting a prokaryote or a eukaryote as the etiologic agent, both of which are featured in this document. Microbes tend to be uniquely suited to adapt to environments where antibiotics and host responses apply pressures that encourage their survival. A laboratory instrument may or may not detect those mutations, which can present a challenge to clinical interpretation. Clearly, microbes grow, multiply, and die very quickly. If any of those events occur during the preanalytical specimen management processes, the results of analysis will be compromised and interpretation could be misleading.
Physicians and other advanced practice providers need confidence that the results provided by the microbiology laboratory are accurate, significant, and clinically relevant. Anything less is below the community standard of care for laboratories. To provide that level of quality, however, the laboratory requires that all microbiology specimens be properly selected, collected, and transported to optimize analysis and interpretation. Because result interpretation in microbiology depends entirely on the quality of the specimen submitted for analysis, specimen management cannot be left to chance, and those that collect specimens for microbiologic analysis must be aware of what the physician needs for patient care as well as what the laboratory needs to provide accurate results, including ensuring that specimens arrive at the laboratory for analysis as quickly as possible after collection (Table 1).
At an elementary level, the physician needs answers to 3 very basic questions from the laboratory: Is my patient's illness caused by a microbe? If so, what is it? What is the susceptibility profile of the organism so therapy can be targeted? To meet those needs, the laboratory requires a specimen that has been appropriately selected, collected, and transported to the laboratory for analysis. Caught in the middle, between the physician and laboratory requirements, are the medical personnel who actually select and collect the specimen and who may not know or understand what the physician or the laboratory needs to do their work. Enhancing the quality of

the specimen is everyone's job, so communication between the physicians, nurses, and laboratory staff should be encouraged and open with no punitive motive or consequences.
The diagnosis of infectious disease is best achieved by applying in-depth knowledge of both medical and laboratory science along with principles of epidemiology and pharmacokinetics of antibiotics and by integrating a strategic view of host–parasite interactions. Clearly, the best outcomes for patients are the result of strong partnerships between the clinician and the microbiology specialist. This document illustrates and promotes this partnership and emphasizes the importance of appropriate specimen management to clinical relevance of the results. One of the most valuable laboratory partners in infectious disease diagnosis is the certified microbiology specialist, particularly a specialist certified as a Diplomate by the American Board of Medical Microbiology, the American Board of Pathology, or the American Board of Medical Laboratory Immunology or their equivalent certified by other organizations. Clinicians should recommend and medical institutions should provide this kind of leadership for the microbiology laboratory or provide formal access to this level of laboratory expertise through consultation.

IMPACT OF SPECIMEN MANAGEMENT ::: EXECUTIVE SUMMARY:
Microbiology specimen selection and collection are the responsibility of the medical personnel, not usually the laboratory, although the certified specialist may be called upon for consultation or assistance. The impact of proper specimen management on patient care is enormous. It is the key to accurate laboratory diagnosis and confirmation, it directly affects patient care and patient outcomes, it influences therapeutic decisions, it impacts hospital infection control, patient length of stay, hospital and laboratory costs, it influences antibiotic stewardship, and it drives laboratory efficiency. Clinicians and other medical personnel should consult the laboratory to ensure that selection, collection, transport, and storage of patient specimens they collect are managed properly.

TENETS OF SPECIMEN MANAGEMENT ::: EXECUTIVE SUMMARY:
Throughout the text, there will be caveats that are relevant to specific specimens and diagnostic protocols for infectious disease diagnosis. However, there are some strategic tenets of specimen management and testing in microbiology that stand as community standards of care and that set microbiology apart from other laboratory departments such as chemistry or hematology.

TEN POINTS OF IMPORTANCE ::: EXECUTIVE SUMMARY:
The microbiology laboratory policy manual should be available at all times for all medical personnel to review or consult and it would be particularly helpful to encourage the nursing staff to review the specimen collection and management portion of the manual. This can facilitate collaboration between the laboratory, with the microbiology expertise, and the specimen collection personnel, who may know very little about microbiology or what the laboratory needs to establish or confirm a diagnosis.
It is important to welcome and actively engage the microbiology laboratory as an integral part of the healthcare team and encourage the hospital or the laboratory facility to have board-certified laboratory specialists on hand or available to optimize infectious disease laboratory diagnosis.

HOW TO USE THIS DOCUMENT ::: EXECUTIVE SUMMARY:
This document is organized by body system, although many organisms are capable of causing disease in >1 body system. There may be a redundant mention of some organisms because of their propensity to infect multiple sites. One of the unique features of this document is its ability to assist clinicians who have specific suspicions regarding possible etiologic agents causing a specific type of disease. When the term "clinician" is used throughout the document, it also includes other licensed, advanced practice providers. Another unique feature is that in most chapters, there are targeted recommendations and precautions regarding selecting and collecting specimens for analysis for a disease process. It is very easy to access critical information about a specific body site just by consulting the table of contents. Within each chapter, there is a table describing the specimen needs regarding a variety of etiologic agents that one may suspect as causing the illness. The test methods in the tables are listed in priority order according to the recommendations of the authors and reviewers.
When room temperature is specified for a certain time period, such as 2 hours, it is expected that the sample should be refrigerated after that time unless specified otherwise in that section.
Almost all specimens for virus detection should be transported on wet ice and frozen at –80°C if

testing is delayed >48 hours, although specimens in viral transport media may be transported at room temperature when rapid (<2 hours) delivery to the laboratory is assured.

HISTORY AND REQUEST:
This document has been endorsed by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). This is not an official guideline of the IDSA but rather an authoritative guide with recommendations for utilizing the microbiology laboratory in infectious disease diagnosis. It is a collaborative effort between clinicians and laboratory experts focusing on optimum use of the laboratory for positive patient outcomes. When the term “recommended” is used in this document, it is not a “graded” recommendation as would be found in a guideline, but rather the preferred or indicated approach for use or application. Future modifications of the document are to be expected, as diagnostic microbiology is a dynamic and rapidly changing discipline. Pediatric parameters have been updated in concordance with Pediatric Clinical Practice Guidelines and Policies, 16th ed., and The Red Book (2015), both published by the American Academy of Pediatrics. Comments and recommendations have been integrated into the appropriate sections.

A. Bloodstream Infections and Infective Endocarditis ::: I. BLOODSTREAM INFECTIONS AND INFECTIONS OF THE CARDIOVASCULAR SYSTEM:
The diagnosis of bloodstream infections (BSIs) is one of the most critical functions of clinical microbiology laboratories. For the great majority of etiologic agents of BSIs, conventional blood culture methods provide positive results within 48 hours; incubation for >5 days seldom is required when modern automated continuous-monitoring blood culture systems and media are used [1, 2]. This includes recovery of historically fastidious organisms such as HACEK [1] (Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, and Kingella) bacteria and Brucella species (spp) [3, 4]. Some microorganisms, such as mycobacteria and dimorphic fungi, require longer incubation periods; others may require special culture media or non-culture-based methods. Although filamentous fungi often require special broth media or lysis-centrifugation vials for detection, most Candida spp grow very well in standard blood culture broths unless the patient has been on antifungal therapy. Unfortunately, blood cultures from patients with suspected candidemia do not yield positive results in almost half of patients. Table 2 provides a summary of diagnostic methods for most BSIs.
For most etiologic agents of infective endocarditis, conventional blood culture methods will suffice [3–5]. However, some less common etiologic agents cannot be detected with current blood culture methods. The most common etiologic agents of culture-negative endocarditis, Bartonella spp and Coxiella burnetii, often can be detected by conventional serologic testing. However, molecular amplification methods may be needed for detection of these organisms as well as others (eg, Tropheryma whipplei, Bartonella spp). In rare instances of culture-negative endocarditis, 16S polymerase chain reaction (PCR) and DNA sequencing of valve tissue may help determine an etiologic agent.
The volume of blood that is obtained for each blood culture request (also known as a blood culture set, consisting of all bottles procured from a single venipuncture or during one catheter draw) is the most important variable in recovering bacteria and fungi from adult and pediatric patients with bloodstream infections [1, 2, 5, 6]. For adults, 20–30 mL of blood per culture set (depending on the manufacturer of the instrument) is recommended and may require >2 culture bottles depending on the system. For neonates and adolescents, an age- and weight- appropriate volume of blood should be cultured (see Table 3 below for recommended volumes). A second important determinant is the number of blood culture sets performed during a given septic episode. Generally, in adults with a suspicion of BSI, 2–4 blood culture sets should be obtained in the evaluation of each septic episode [5, 7].
The timing of blood culture orders should be dictated by patient acuity. In urgent situations, 2 or more blood culture sets can be obtained sequentially over a short time interval (minutes), after which empiric therapy can be initiated. In less urgent situations, obtaining blood culture sets may be spaced over several hours or more.
Skin contaminants in blood culture bottles are common, very costly to the healthcare system, and frequently confusing to clinicians. To minimize the risk of contamination of the blood culture with commensal skin microbiota, meticulous care should be taken in skin preparation prior to venipuncture. In addition, new products are now available that allow diversion and discard of the first few milliliters of blood that are most likely to contain skin contaminants. Consensus guidelines [2] and expert panels [1] recommend peripheral venipuncture as the preferred

technique for obtaining blood for culture based on data showing that blood obtained in this fashion is less likely to be contaminated than blood obtained from an intravascular catheter or other device. Several studies have documented that iodine tincture, chlorine peroxide, and chlorhexidine gluconate (CHG) are superior to povidone-iodine preparations as skin disinfectants for blood culture [1, 2]. Iodine tincture and CHG require about 30 seconds to exert an antiseptic effect compared with 1.5–2 minutes for povidone-iodine preparations [2]. Two recent studies have documented equivalent contamination rates with iodine tincture and CHG [8, 9]. CHG is not recommended for use in infants <2 months of age but povidone-iodine followed by alcohol is recommended.

Blood cultures contaminated with skin flora during collection are common but contamination rates should not exceed 3%. Laboratories should have policies and procedures for abbreviating the workup and reporting of common blood culture contaminants (eg, coagulase-negative staphylococci, viridans group streptococci, diphtheroids, Bacillus spp other than B. anthracis). These procedures may include abbreviated identification of the organism, absence of susceptibility testing, and a comment that instructs the clinician to contact the laboratory if the culture result is thought to be clinically significant and requires additional workup and susceptibility results.

Physicians should expect to be called and notified by the laboratory every time a blood culture becomes positive since these specimens often represent life-threatening infections. If the physician wishes not to be notified during specific times, arrangements must be made by the physician for a delegated healthcare professional to receive the call and relay the report.

Key points for the laboratory diagnosis of bacteremia/fungemia:

B. Infections Associated With Vascular Catheters ::: I. BLOODSTREAM INFECTIONS AND INFECTIONS OF THE CARDIOVASCULAR SYSTEM:

The diagnosis of catheter-associated BSIs is often one of exclusion, and a microbiologic gold standard for diagnosis does not exist. Although a number of different microbiologic methods have been described, the available data do not allow firm conclusions to be made about the relative merits of these various diagnostic techniques [10–12]. Fundamental to the diagnosis of catheter-associated BSI is documentation of bacteremia. The clinical significance of a positive culture from an indwelling catheter segment or tip in the absence of positive blood cultures is unknown. The next essential diagnostic component is demonstrating that the infection is caused by the catheter. This usually requires exclusion of other potential primary foci for the BSI. Some investigators have concluded that catheter tip cultures have such poor predictive value that they should not be performed [13].

Numerous diagnostic techniques for catheter cultures have been described and may provide adjunctive evidence of catheter-associated BSI; however, all have potential pitfalls that make interpretation of results problematic. Routine culture of intravenous catheter tips at the time of catheter removal has no clinical value and should not be done [13]. Although not performed in most laboratories, the methods described include the following:

C. Infected (Mycotic) Aneurysms and Vascular Grafts ::: I. BLOODSTREAM INFECTIONS AND INFECTIONS OF THE CARDIOVASCULAR SYSTEM:

Infected (mycotic) aneurysms and infections of vascular grafts may result in positive blood cultures. Definitive diagnosis requires microscopic visualization and/or culture recovery of etiologic agents from representative biopsy or graft material (Table 4).

D. Pericarditis and Myocarditis ::: I. BLOODSTREAM INFECTIONS AND INFECTIONS OF THE CARDIOVASCULAR SYSTEM:

Numerous viruses, bacteria, rickettsiae, fungi, and parasites have been implicated as etiologic agents of pericarditis and myocarditis. In many patients with pericarditis and in the overwhelming majority of patients with myocarditis, an etiologic diagnosis is never made and patients are treated empirically. In selected instances when it is important clinically to define the specific cause of infection, a microbiologic diagnosis should be pursued aggressively. Unfortunately, however, the available diagnostic resources are quite limited, and there are no firm diagnostic guidelines that can be given. Some of the more common and clinically important pathogens are listed in Table 5 below. When a microbiologic diagnosis of less common etiologic agents is required, especially when specialized techniques or methods are necessary, consultation with the laboratory director should be undertaken. There is considerable overlap between pericarditis and myocarditis with respect to both etiologic agents and disease manifestations.

A. Meningitis ::: II. CENTRAL NERVOUS SYSTEM INFECTIONS:
The most common etiologic agents of acute meningitis are viruses (echoviruses and parechoviruses) and bacteria (Streptococcus pneumoniae and Neisseria meningitidis) (Table 6). Patient age and other factors (ie, immunostatus, having undergone neurosurgery, trauma) are associated with specific pathogens.
Molecular testing has replaced viral culture for the diagnosis of enteroviral meningitis, but is not routinely relied on for the detection of bacteria in CSF where Gram stain and bacterial culture should be ordered. The sensitivity of the Gram stain for the diagnosis of bacterial meningitis is 60%–80% in patients who have not received antimicrobial therapy and 40%–60% in patients who have received treatment [21]. Bacterial antigen testing on CSF is no longer recommended and should not be ordered nor should the laboratory provide this service. Early, incorrect assumptions held that selected antigen tests on CSF may have some value in patients who received therapy prior to specimen collection with negative Gram stain and negative culture results [22], but this is no longer recommended. In patients suspected of having bacterial meningitis, at least 2–4 blood cultures should be performed, but therapy should not be delayed.
Organisms expected to cause chronic meningitis (symptoms lasting ≥4 weeks) include Mycobacterium tuberculosis, fungi, and spirochetes (Table 6). Because the sensitivity of nucleic acid amplification tests (NAAT) for M. tuberculosis in nonrespiratory specimens may be poor, culture should also be requested [20, 23]. The reported sensitivity of culture for diagnosing tuberculous meningitis is 25%–70% [24]. The highest yields for acid-fast bacilli (AFB) smear and AFB culture occur when large volumes (≥5 mL) of CSF are used to perform the testing. The cryptococcal antigen test has replaced the India ink stain for rapid diagnosis of meningitis caused by Cryptococcus neoformans or Cryptococcus gattii and should be readily available in most laboratories. This test is most sensitive when performed on CSF rather than serum. The sensitivity and specificity of cryptococcal antigen tests are >90%, but false-negative and false-positive results may occur, for example in patients with human immunodeficiency virus (HIV)/AIDS. Complement fixation test performed on CSF is recommended for the diagnosis of coccidioidal meningitis since direct fungal smear and culture are often negative. Detection of Coccidioides antibody in CSF by immunodiffusion has lower specificity than complement fixation.

B. Encephalitis ::: II. CENTRAL NERVOUS SYSTEM INFECTIONS:
Encephalitis is an infection of the brain parenchyma causing abnormal cerebral function (altered mental status, behavior or speech disturbances, sensory or motor deficits). Despite advancements in molecular technology for the diagnosis of CNS infections, the etiologic agent of encephalitis often cannot be identified. The California Encephalitis Project identified a definite or probable etiologic agent for only 16% of 1570 immunocompetent patients enrolled from 1998 to 2005 (69% viral, 20% bacterial, 7% prion, 3% parasitic, 1% fungal); a possible cause was identified for an additional 13% of patients [25]. Immunostatus, travel, and other exposure history (insects, animals, water, sexual) should guide testing. The Infectious Diseases Society of America (IDSA) practice guidelines provide a detailed listing of risk factors associated with specific etiologic agents [26].
Although the diagnosis of a specific viral cause is usually based on testing performed on CSF, testing of specimens collected from other sites may be helpful. The virus most commonly identified as causing encephalitis is herpes simplex virus (HSV) with 90% HSV-1. The sensitivity and specificity of NAAT for HSV encephalitis are >95%; early data showed that HSV is cultured from CSF in <5% of cases [27, 28]. Reports of false-negative HSV NAAT are the basis of recommendations to collect another CSF specimen 3–7 days later for repeat testing if HSV encephalitis continues to be suspected [26, 29]. The sensitivity of NAAT performed on CSF for enterovirus encephalitis is >95% and the sensitivity of culture is 65%–75% (recovery from throat or stool is circumstantial etiologic evidence) [27]. Additional NAAT specific for parechoviruses is recommended for young children [29]. Because the performance characteristics of molecular testing for other causes of viral encephalitis are not well established, serology and repeat molecular testing may be required (Table 7).

C. Focal Infections of Brain Parenchyma ::: II. CENTRAL NERVOUS SYSTEM INFECTIONS:
Focal parenchymal brain infections start as cerebritis, then progress to necrosis surrounded by a fibrous capsule. There are 2 broad categories of pathogenesis: (1) contiguous spread (otitis media, sinusitis, mastoiditis, and dental infection), trauma, neurosurgical complication, or (2) hematogenous spread from a distant site of infection (skin, pulmonary, pelvic, intra-abdominal,

esophageal, endocarditis). A brain abscess in an immunocompetent host is usually caused by bacteria (Table 8). A wider array of organisms is encountered in immunocompromised individuals.

D. Central Nervous System Shunt Infections ::: II. CENTRAL NERVOUS SYSTEM INFECTIONS:
Shunts are placed to divert CSF for the treatment of hydrocephalus. The proximal portion is placed in a cerebral ventricle, intracranial cyst, or the subarachnoid space (lumbar region). The distal portion may be internalized (peritoneal, vascular, or pleural space) or externalized. Five to 15% of shunts become infected (Table 9). Potential routes of shunt infection include contamination at time of placement, contamination from the distal portion (retrograde), breakdown of the skin over the shunt, and hematogenous seeding. Blood cultures should also be collected if the shunt terminates in a vascular space (ventriculoatrial shunt). Most CNS shunt infections are caused by bacteria. Fungi are more likely to cause shunt infections in immunocompromised patients and those receiving total parenteral nutrition, steroids, or broad-spectrum antibiotics. Culture of shunt or drain components after removal should not be performed unless the patient has symptoms of a CNS infection [35].

E. Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis ::: II. CENTRAL NERVOUS SYSTEM INFECTIONS:
Cranial subdural empyema and cranial epidural abscess are neurosurgical emergencies that are usually caused by bacteria (streptococci, staphylococci, aerobic gram-negative bacilli, anaerobes, often polymicrobial) (Table 10). Mycobacteria and fungi are rare causes. Predisposing conditions include sinusitis, otitis media, mastoiditis, neurosurgery, head trauma, subdural hematoma, and meningitis (infants).
The pathogenesis of spinal epidural abscess includes hematogenous spread (skin, urinary tract, mouth, mastoid, lung infection), direct extension (vertebral osteomyelitis, discitis), trauma, or postprocedural complication (surgery, biopsy, lumbar puncture, anesthesia). Spinal epidural abscess is usually caused by staphylococci, streptococci, aerobic gram-negative bacilli, and anaerobes. Nocardia spp, mycobacteria, and fungi may also cause spinal epidural abscess. Spinal subdural empyema is similar to spinal epidural abscess in clinical presentation and causative organisms.
Magnetic resonance imaging is the optimal diagnostic procedure for suppurative intracranial thrombophlebitis. The etiologic agent may be recovered from cerebrospinal fluid and blood cultures. Causative organisms are similar to cranial epidural abscess and cranial subdural empyema. Empiric antimicrobial therapy is usually based on the predisposing clinical condition.

A. Specimen Collection, Processing, and Transport ::: III. OCULAR INFECTIONS:
Because ocular infections may involve one or both eyes and etiologies may differ, clinicians must clearly mark specimens as to which eye has been sampled, especially in those patients who have bilateral disease.
Collection of specimens from anatomical structures surrounding the eye is typically done using swabs (Table 11). The most commonly collected specimens are from the conjunctiva. Cultures for aerobic bacteria and detection of Chlamydia and viruses either by culture or NAAT are most commonly performed, although none are as yet FDA approved for detection in eye specimens. Since direct microscopic examination may be useful in preliminary diagnosis of conjunctivitis, obtaining dual swabs, one for culture and one for smear preparation, is recommended. Smears may be made for Gram stain, calcofluor stain for fungi and Acanthamoeba, or direct fluorescent antibody (DFA) for Chlamydia trachomatis. Appropriate transport media should be provided by the laboratory and available at the collection site for specimens submitted for Chlamydia and/or viral culture or NAAT [36]. Although NAAT tests are preferred for the diagnosis of viral ocular infections because of their increased sensitivity and more rapid turnaround time, if viral culture is requested, specimens should be submitted on ice using viral transport medium, especially if specimen transport is prolonged [36].
Specimens obtained from either the surface or the globe of the eye are almost always collected by ophthalmologists. Specimen types include swabs of ulcers, corneal scrapings, impression membrane cultures, biopsies, or anterior chamber aspirates, or vitreous aspirates/washings [36, 37]. The volume of specimens is always limited. This specimen limitation makes it necessary for the laboratory to prioritize procedures depending on what organisms are sought; this should always be done after discussion with the ophthalmologist who collects the specimen and the infectious disease consultant when appropriate. This is particularly important because all major pathogen groups—viruses, parasites, bacteria, mycobacteria, and fungi—can cause ocular

infection. Both epidemiology and clinical presentation are used to narrow the organism(s) sought and the laboratory tests requested. Because of the limited specimen size seen with scrapings and biopsies, the laboratory and ophthalmologist may agree to inoculate these specimens onto media and prepare smears at the bedside. In this case, the laboratory should supply the necessary media and slides to the ophthalmologist. If these supplies are stored in the clinic or operating suite for ready access by the surgeon, it is the laboratory's responsibility to assure that these materials do not out-date and meet all quality control standards. Aspirates from the anterior chamber or vitreous are the optimal specimens for detection of anaerobic bacteria and viral agents; they can be submitted in syringes with needles removed. Syringes should be placed in a leak-proof outer container for transport. Injection of the fluid into a small sterile vial (provided by the laboratory) is preferable. The same principles for specimen collection and transport described for conjunctival specimens apply to these specimens as well.

B. Orbital and Periorbital Cellulitis ::: III. OCULAR INFECTIONS:
Orbital cellulitis is almost always a complication of sinusitis and the organisms associated with it include Streptococcus pneumoniae, nontypeable Haemophilus influenzae, Streptococcus pyogenes, Moraxella spp, anaerobic bacteria, Aspergillus spp, and the Mucorales (formerly Zygomycetes). Periorbital cellulitis usually arises as a result either of localized trauma or bacteremia most often caused by Staphylococcus aureus, S. pyogenes, or S. pneumoniae [38]. Diagnosis of these infections is either based on positive blood cultures or, in the case of orbital cellulitis, culture of drainage material aspirated from the subperiosteal region of the sinuses.

C. Infection of the Eyelids and Lacrimal System ::: III. OCULAR INFECTIONS:
Blepharitis, canaliculitis, and dacryocystitis are all superficial infections that are generally self-limited. The organisms associated with these infections are predominantly gram-positive bacteria, although various gram-negative bacteria, anaerobes, and fungi all have been recovered [39]. A limitation of many studies of these infections is that microbiologic data on control populations are frequently lacking. The organisms commonly recovered are part of the indigenous skin microflora such as coagulase negative staphylococci and diphtheroids, so attributing a pathogenic role to these organisms in these conditions is difficult. Cultures from these sites are rarely submitted for diagnostic workup. If cultures for canaliculitis are considered, concretions recovered during canalicular compression or canaliculotomy are recommended. Strategies for the diagnosis of these superficial infections should be similar to those for conjunctivitis.

D. Conjunctivitis ::: III. OCULAR INFECTIONS:
Most cases of conjunctivitis are caused by bacteria or viruses that are typically associated with upper respiratory tract infections [40, 41]. Because of the distinctive clinical presentation of both bacterial and viral conjunctivitis coupled with the self-limited nature of these infections, determining its etiology is infrequently attempted [42]. When tests are requested, diagnosis of bacterial conjunctivitis is often compromised by the prior use of empiric antibacterial therapy [40, 41]. Sexually active patients who present with bacterial conjunctivitis should have an aggressive diagnostic workup with Gram stain and cultures because of their risk for N. gonorrhoeae conjunctivitis [43]. This is a sight-threatening infection which can result in perforation of the globe. In the developing world, trachoma, a form of conjunctivitis due to specific strains of C. trachomatis is a leading cause of blindness, especially in children [44]. Off-label use of commercial NAAT assays is used for detection of this agent in research settings [44]. Certain organisms that are part of the indigenous skin and mucous membrane microflora, such as coagulase-negative staphylococci, Corynebacterium spp, and viridans streptococci, are generally considered nonpathogenic when recovered from the conjunctival mucosa and are considered to be "normal flora." In specimens taken from the surface or interior of the eye, these organisms along with Cutibacterium (Propionibacterium) acnes are considered pathogens, especially in patient postcataract or LASIK surgery [36]. Adenovirus, the etiologic agent of "pink eye," is highly transmissible in a variety of settings. This is almost always a clinical diagnosis, although for epidemiologic purposes culture or NAAT can be done [36]. Most cases of neonatal conjunctivitis are due to Neisseria gonorrhoeae, C. trachomatis, or herpes simplex virus. Commercial NAATs for both N. gonorrhoeae and C. trachomatis are not FDA approved for this specimen type so culture or in the case of C. trachomatis, DFA testing, if available, can be used [36, 44]. Pseudomonas aeruginosa is a rare but life-threatening cause of neonatal conjunctivitis in hospitalized infants.

E. Keratitis ::: III. OCULAR INFECTIONS:

Corneal infections usually occur in 3 distinct patient populations: those with ocular trauma with foreign objects, those with postsurgical complications of corneal surgery, and in patients who practice poor hygiene associated with their extended-wear contact lenses [45, 46].
Postvaccination keratitis is a well-recognized complication of vaccinia vaccination and should be considered in the appropriate clinical setting [47]. Corneal infections can also result from reactivation of herpes viruses including HSV and varicella zoster virus [48]. It is important to note that the use of dyes and topical anesthetics may inhibit NAAT reactions used to diagnose keratitis [48]. The eye surface should be thoroughly rinsed with nonbacteriostatic saline before specimens for NAATs are obtained [48, 49] (Table 12).
The most common corneal infections occur in patients who improperly use their contact lens system. Because these patients are usually treated with antimicrobial agents prior to obtaining specimens for bacterial cultures, some ophthalmologists favor culturing contact lens solution and cases. However, culture of such solutions and cases is not recommended because of the frequency with which they are falsely positive [50, 51]. Pseudomonas aeruginosa is the most common cause of sporadic contact lens–associated keratitis, but outbreaks of keratitis due to contamination of contact lens care solutions have been recently reported with both Fusarium and Acanthamoeba [50–53]. Sporadic cases of Acanthamoeba keratitis are increasing, with >90% associated with improper contact lens use [54]. Postsurgical keratitis infections are frequently due to either coagulase-negative staphylococci or C. acnes, so in this setting these organisms should not be considered contaminants but as potential pathogens [36]. Keratitis postcorneal transplant is most commonly due to Candida spp (80% of cases). This is due in part to the most widely used corneal holding medium not containing any antifungal agents [55].
Keratitis following trauma due to foreign objects is frequently caused by organisms found in the environment. Included in this group are environmental gram-negative rods such as P. aeruginosa, Nocardia spp, molds including dematiaceous fungi, and environmental mycobacteria [36].
Corneal biopsies are recommended in patients in whom keratitis persists or worsens. In a small series (n = 48), organism was found in 44% who had negative corneal scrapings. However, most pathogens were detected by histopathology (n = 19) and not culture (n = 9) [56]. Acanthamoeba sp (n = 8) and fungi (n = 6) represented most of the organisms detected by histopathology.

F. Endophthalmitis ::: III. OCULAR INFECTIONS:
Endophthalmitis can arise either by exogenous introduction of pathogens into the eye following trauma or surgery, or as a result of endogenous introduction of pathogens across the blood–eye barrier. Depending upon the mode of pathogenesis, the spectrum of causative agents will vary (Table 13). Specimens for diagnosis of endophthalmitis can be obtained by aspiration of aqueous fluid or vitreous fluid/washing or via biopsy [57–59]. Specimen amounts of both aqueous and vitreous fluid are small, so discretion must be exercised in determining for which agents the specimen should be examined. Alternatively, vitrectomy, a surgical procedure, allows collections of comparatively large fluid volumes (>5 mL) by “washing” the vitreous with a nonbacteriostatic balanced salt solution [58, 59] or by membrane filtration.
Postoperative endophthalmitis is most often caused by gram-positive organisms with coagulase-negative staphylococci predominating; chronic postoperative endophthalmitis can be due to C. acnes, so this organism should not be routinely dismissed as a contaminant [58–61]. Postcorneal endophthalmitis is due primarily to Candida spp (65%) and gram-positive organisms (33%), with Candida and the majority of the gram-positive organism resistant to the antimicrobials present in the cornea holding medium [56].
Environmental organisms such as dematiaceous fungi, Fusarium spp, Bacillus cereus, Nocardia spp, Mycobacterium chelonae, and glucose-fermenting gram-negative rods are more commonly encountered in patients with exogenous endophthalmitis [61]. Endogenous endophthalmitis, because of its association with bacteremia and fungemia, is usually caused by those organisms most responsible for BSIs; for example, Candida albicans and related species, Aspergillus spp, S. aureus, S. pneumoniae, the Enterobacteriaceae (especially Klebsiella pneumoniae), and P. aeruginosa [62, 63]. Viruses and parasites are rarely found to cause endophthalmitis; however, as in cases of trauma or severe immunosuppression, infection due to agents such as the herpes viruses, Toxoplasma gondii, Toxocara spp, Echinococcus spp, and Onchocerca volvulus do occur [64, 65] and typically involve the uvea and retina. For further information on the diagnosis of ocular infections caused by O. volvulus, see Section XV-C.

G. Uveitis/Retinitis ::: III. OCULAR INFECTIONS:

The inflammation characteristic of uveitis/retinitis is typically due to either autoimmune conditions or is idiopathic [66]. Only infrequently is it due to infection, which is almost always caused by endogenous microbes accessing the eye via a breach in the blood–eye barrier. Because uveitis and retinitis, like endogenous endophthalmitis, are localized manifestations of systemic infections, diagnosis of the etiology of systemic infections should be coupled with a careful ocular examination, preferably performed by an ophthalmologist with specific infectious disease expertise. Important causes of uveitis/retinitis include T. gondii, cytomegalovirus (CMV), HSV, varicella zoster virus, M. tuberculosis, and Treponema pallidum [64–67]. Toxocara canis and rubella are additional agents to be considered in pediatrics.

Toxoplasma gondii is the most common infectious cause of retinitis. Diagnosis is typically made on clinical grounds supported by serology. In the industrialized world, the presence of T. gondii IgG lacks specificity for the diagnosis of ocular toxoplasmosis; therefore, serology is only valuable in the setting of acute infection or when the patient has an ocular examination pathognomonic for toxoplasmosis, demonstrating retinochoroiditis in a majority of cases. The comparison of intraocular antibody levels in aqueous humor to that in serum has been found to be a useful means for diagnosing ocular toxoplasmosis, although not consistently accurate. Because the specimen needed for testing can only be obtained by an experienced ophthalmologist and is an invasive procedure, it is unlikely that this technique will be used outside the research setting [68]. NAAT of blood, vitreous, or aqueous fluids is not as sensitive as intraocular antibody determinations, but the specimens for testing may be more easily obtained. Sensitivities of NAATs ranging from 50% to 80% have been reported in patients with T. gondii retinitis depending upon the sequence used and the specimen tested. It should be noted that the total numbers of specimens tested in these studies are small but there is increasing evidence to the diagnostic value of NAAT in T. gondii retinitis [68–72].
Since the advent of highly active antiretroviral treatment, CMV retinitis has become much less frequent. Nevertheless, cases do occur in HIV patients who have either failed HIV therapy or as an AIDS-presenting diagnosis [73]. In addition, CMV retinitis has been a well-recognized complication of bone marrow and solid organ transplantation, less frequent recently due to improvements in preemptive detection and therapy. CMV retinitis is frequently diagnosed clinically because of characteristic lesions seen on ophthalmologic examination. Quantitative CMV NAAT performed on peripheral blood is also a useful tool in the diagnosis and management of this infection. Patients with detectable CMV viral loads have a higher likelihood of retinal disease progression, and those with high CMV viral loads have increased mortality. Patients with undetectable CMV viral loads have a low likelihood of having virus that is resistant to antiviral agents [74]. Because of interlaboratory variation in viral quantification, what represents a positive CMV viral load and a high CMV viral load will vary among laboratories [75]. Physicians should consult the laboratory performing the CMV viral load for assistance with test interpretation.
Patients with ocular syphilis may present with normal CSF or may frequently have CNS findings associated with either acute syphilitic meningitis or neurosyphilis. Patients with syphilitic uveitis typically have high rapid plasma reagin (RPR) titers [67]. Cell counts, total protein, and glucose, along with Venereal Disease Research Laboratory (VDRL) testing of CSF, are recommended in clinical settings where syphilitic uveitis is suspected [67].
Finally, metagenomics analysis is beginning to be applied in research settings for the diagnosis of unusual cases of uveitis. This diagnostic approach is likely to be available for the diagnosis of endophthalmitis, uveitis, and retinitis in the near future [76].

A. Otitis Media ::: V. UPPER RESPIRATORY TRACT BACTERIAL AND FUNGAL INFECTIONS:
Otitis media (OM) is the single most frequent condition causing pediatric patients to be taken to a healthcare provider and to be given antibiotics [86]. Acute OM with effusion is the clinical variant of OM most likely to have a bacterial etiology and, as a result, most likely to benefit from antimicrobial therapy [87, 88] (Table 16). Streptococcus pneumoniae, nontypeable Haemophilus influenzae, and Moraxella catarrhalis are the most common bacterial causes of OM, with S. aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa occurring less commonly [89]. Turicella otitidis and Staphylococcus auricularis are emerging pathogens thought to cause OM, but additional studies are needed to determine the true significance of these organisms [89, 90].
Chronic suppurative OM is associated with a higher rate of complications than acute OM. Pseudomonas aeruginosa and S. aureus are the most common pathogens in chronic OM [91]. A variety of respiratory viruses are known to cause OM; however, there exists no pathogen specific therapy and as a result, there is little reason to attempt to establish an etiologic diagnosis in

patients with a viral etiology. Efforts to determine the cause of OM are best reserved for patients likely to have a bacterial etiology (recent onset, bulging tympanic membrane, pain, or exudate) who have not responded to prior courses of antimicrobial therapy, patients with immunological deficiencies, and acutely ill patients [86, 88]. The only representative specimen is middle ear fluid obtained either by tympanocentesis or, in patients with otorrhea or myringotomy tubes, by collecting drainage on mini-tipped swabs directly after cleaning the ear canal. Cultures of the pharynx, nasopharynx, anterior nares, or nasal drainage material are of no value in attempting to establish an etiologic diagnosis of bacterial OM (Table 16) [92].

B. Sinusitis ::: V. UPPER RESPIRATORY TRACT BACTERIAL AND FUNGAL INFECTIONS:
Rhinosinusitis (the preferred term encompassing both acute and chronic disease) affects approximately 12%–15.2% of the adult population in the United States annually. The direct costs of managing acute and chronic rhinosinusitis exceed US$11 billion per year. The etiological agents of rhinosinusitis vary based upon the duration of symptoms and whether it is community acquired or of nosocomial origin (Table 17). Streptococcus pneumoniae, nontypeable H. influenzae, and Moraxella catarrhalis are the most common bacterial causes of acute maxillary sinusitis. The role of respiratory viruses in sinusitis needs further study, but most patients with acute sinusitis have an upper respiratory virus detectable early in the illness [93]. Staphylococcus aureus, gram-negative bacilli, Streptococcus spp, and anaerobic bacteria are associated more frequently with subacute, chronic, or healthcare-associated sinusitis [94]. The role of fungi as etiological agents is more controversial, possibly due to numerous publications that used poor sample collection methods and thus did not recover the fungal agents. In immunocompetent hosts, fungi are associated most often with chronic sinusitis, though the significance of fungal presence in chronic sinusitis is frequently uncertain [93, 95, 96]. Invasive sinusitis due to fungal infections in severely immunocompromised persons or uncontrolled diabetic patients is often severe and carries a high mortality rate.
Attempts to establish an etiologic diagnosis of sinusitis are typically reserved for patients with complicated infections or chronic disease (patients who are seriously ill, immunocompromised, continue to deteriorate clinically despite extended courses of antimicrobial therapy, or have recurrent bouts of acute rhinosinusitis with clearing between episodes). Swabs are not recommended for collecting sinus specimens since an aspirate is much more productive of the true etiologic agent(s) and is the specimen of choice. Endoscopically obtained swabs can recover bacterial pathogens but rarely detect the causative fungi [92, 97, 98]. In maxillary sinusitis, antral puncture with sinus aspiration (though seldom done) and, in adults, swabs of material draining from the middle meatus obtained under endoscopic guidance represent the only adequate specimens. Cultures of middle meatus drainage specimens are not recommended for pediatric patients due to colonization with normal microbiota, which overlaps with potential respiratory tract pathogens. Examination of nasal drainage material is of no value in attempting to determine the cause of maxillary sinusitis. Surgical procedures are necessary to obtain specimens representative of infection of the frontal, sphenoid, or ethmoid sinuses. To establish a fungal etiology, an endoscopic sinus aspirate is recommended [98] but is often unproductive for a fungal agent. Tissue biopsy may be more productive.

C. Pharyngitis ::: V. UPPER RESPIRATORY TRACT BACTERIAL AND FUNGAL INFECTIONS:
Acute pharyngitis accounts for roughly 1.3% of outpatient visits to healthcare providers in the United States and was responsible for 15 million patient visits in 2006 [99]. Most pharyngitis is viral and need not be treated, but 10%–15% of pharyngitis in adults and 15%–30% in children is due to group A streptococci [100]. Differences between the epidemiology of various infectious agents related to the age of the patient, the season of the year, accompanying signs and symptoms, and the presence or absence of systemic disease are insufficient to establish a definitive etiologic diagnosis on clinical and epidemiologic grounds alone [101]. Consequently, the results of laboratory tests play a central role in guiding therapeutic decisions (Table 18). Antimicrobial therapy is warranted only in patients with pharyngitis with a proven bacterial etiology [102].
Streptococcus pyogenes (group A β-hemolytic Streptococcus) is the most common bacterial cause of pharyngitis and carries with it potentially serious sequelae, primarily in children, if left undiagnosed or inadequately treated. Several laboratory tests, including culture, rapid antigen tests, and molecular methods, have been used to establish an etiologic diagnosis of pharyngitis due to this organism [101, 103]. During the past few decades, rapid antigen tests for S. pyogenes have been used extensively in the evaluation of patients with pharyngitis. Such tests are

technically nondemanding, generally reliable, and often performed at the point of care. For any of these methods, accuracy and clinical relevance depend on appropriate sampling technique. There is a consensus among the professional societies that negative rapid antigen tests for S. pyogenes in children should be confirmed by culture or molecular assay. Although this is generally not necessary for negative test results in adults due to the lower risk of complications, new guidelines suggest that either conventional culture or confirmation of negative rapid antigen test results by culture should be used to achieve maximal sensitivity for diagnosis of S. pyogenes pharyngitis in adults [103]. Laboratories accredited by the College of American Pathologists are required to back up negative rapid antigen tests with culture. Rapid, Clinical Laboratory Improvement Amendments (CLIA)–waived methods for molecular group A Streptococcus testing provide improved sensitivity and may not require culture confirmation [104, 105], though they have not yet been incorporated into consensus guidelines.
The role of non–group A β-hemolytic streptococci, in particular, groups C and G, as causes of pharyngitis is controversial. However, many healthcare providers consider these organisms to be of significance and base therapeutic decisions on their detection. Rare cases of poststreptococcal glomerulonephritis after infection with these species have been reported. Therefore, we have included guidance for detecting group C and G β-hemolytic streptococci (large colony producers, since Streptococcus anginosus group, characteristically yielding pinpoint colonies, does not cause pharyngitis) in pharyngeal swab specimens, but indicate that this should be done only in settings in which these organisms are considered to be of significance, such as outbreaks of epidemiologically associated cases of pharyngitis. Recovery of the same organism from multiple patients during an outbreak should be investigated. Arcanobacterium haemolyticum also causes pharyngitis but less commonly. It occurs most often in teenagers and young adults and causes a highly suggestive scarlatina-form rash in some patients. Neisseria gonorrhoeae and Corynebacterium diphtheriae, in very specific patient and epidemiologic settings, may also cause pharyngitis [100].
Respiratory viruses are the most common cause of pharyngitis in both adult and pediatric populations; however, it is unnecessary to define a specific etiology in patients with pharyngitis due to respiratory viruses since there exists no pathogen-directed therapy for these agents. HSV, HIV, and Epstein-Barr virus (EBV) may also cause pharyngitis. Because of the epidemiologic and clinical implications of infection due to HSV, HIV, and EBV, circumstances may arise in which it is important to attempt to determine if an individual patient's infection is caused by one of these 3 agents [100].
Recent studies have shown a relationship between Fusobacterium necrophorum and pharyngitis in some patients. In this case, throat infection could be a prelude to Lemierre syndrome. Fusobacterium necrophorum is an anaerobic organism and, as such, will require additional media and the use of anaerobic isolation and identification procedures, which most laboratories are not prepared to use with throat specimens. Notify the laboratory of the suspected diagnosis and the etiologic agent so that appropriate procedures can be available. In the absence of anaerobic capability of the laboratory, this would be sent out to a reference laboratory [106–108].

A. Bronchitis and Bronchiolitis ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:

Table 19 lists the etiologic agents and diagnostic approaches for bronchiolitis, acute bronchitis, acute exacerbation of chronic bronchitis, and pertussis, clinical syndromes that involve inflammation of the tracheobronchial tree [109, 110]. Bronchiolitis is the most common lower respiratory infection in children [109, 110]. Viruses, alone or in combination, constitute the major causes of the syndrome characterized by bronchospasm (wheezing) resulting from acute inflammation, airway edema, and increased mucus production [109, 110]. Acute bronchitis is largely due to viral pathogens and is less frequently caused by Mycoplasma pneumoniae and Chlamydia pneumoniae. Pertussis, classically known as whooping cough, caused by Bordetella pertussis, should be considered in an adolescent or young adult with paroxysmal cough. NAATs in combination with culture are the recommended tests of choice for B. pertussis detection. Currently there are a few FDA-cleared platforms for B. pertussis detection. The Centers for Disease Control and Prevention (CDC) has suggested best practices when using molecular tests for pertussis detection (https://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html).

Streptococcus pneumoniae and Haemophilus influenzae do not play an established role in acute bronchitis but they, along with Moraxella catarrhalis, do figure prominently in cases of acute

exacerbation of chronic bronchitis. Several FDA-approved NAAT platforms are available for the detection of a broad range of respiratory viruses and some of the “atypical bacteria” associated with respiratory syndromes. These have largely replaced rapid antigen detection tests and culture in most institutions. Performance characteristics vary among the various panels and singleplex NAATs. Specimen sources may also vary depending upon the assay. Readers should become familiar with the platforms offered in their respective institutions and the approved specimen sources, collection devices, and transport requirements. Respiratory syncytial virus, human rhinovirus, human metapneumovirus, human coronavirus, and type 3 parainfluenza virus are significant causes of bronchiolitis in infants and young children [111]. Coinfections are not uncommon and have been observed in up to 30% of cases. Several molecular panels for the detection of bacterial causes of pneumonia and their resistance markers are currently in clinical trials.

B. Community-Acquired Pneumonia ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:
The diagnosis of CAP is based on the presence of specific symptoms and suggestive radiographic features, such as pulmonary infiltrates and/or pleural effusion. Carefully obtained microbiological data can support the diagnosis, but often fails to provide an etiologic agent. Table 20 lists the more common causes of CAP. Other less common etiologies may need to be considered depending upon recent travel history or exposure to vectors or animals that transmit zoonotic pathogens such as Sin Nombre virus (hantavirus pulmonary syndrome) or Yersinia pestis (pneumonic plague, endemic in the western United States).
The rationale for attempting to establish an etiology is that identification of a pathogen will focus the antibiotic management for a particular patient [112]. In addition, identification of certain pathogens such as Legionella spp, influenza viruses, and the agents of bioterrorism have important public health significance. IDSA/American Thoracic Society (ATS) practice guidelines (currently under revision) consider diagnostic testing as optional for the patient who is not hospitalized [113]. Those patients who require admission should have pretreatment blood cultures, culture and Gram stain of good-quality samples of expectorated sputum and, if disease is severe, urinary antigen tests for S. pneumoniae and Legionella pneumophila where available. The recommendations for children are in agreement with the adult recommendations with respect to when to obtain blood cultures and sputum cultures but differ slightly for other laboratory tests [114]. Testing for viral pathogens is recommended in both outpatient and inpatient settings [114]. Although a weak recommendation, in children with appropriate signs and symptoms, Mycoplasma pneumoniae testing is indicated. There are several molecular assays available for M. pneumoniae detection [114] and at least one assay that also detects Chlamydia pneumoniae. Urinary antigen testing for S. pneumoniae detection is not recommended for use in children because of the poor specificity of the test [114].
Laboratories must have a mechanism in place for screening sputum samples for acceptability (to exclude those that are heavily contaminated with oropharyngeal microbiota and not representative of deeply expectorated samples) prior to setting up routine bacterial culture. Poor-quality specimens provide misleading results and should be rejected because interpretation would be compromised. Endotracheal aspirates or bronchoscopically obtained samples (including “mini–bronchoalveolar lavage” [BAL] using the Combicath [KOL Bio Medical Instruments, Chantilly, Virginia] or similar technology) may be required in the hospitalized patient who is intubated or unable to produce an adequate sputum sample. A thoracentesis should be performed in the patient with a pleural effusion.
Mycobacterial infections should be in the differential diagnosis of CAP that fails to respond to therapy for the typical CAP pathogens. Mycobacterium tuberculosis, although declining in the United States in recent years, is still an important pathogen among immigrant populations. Mycobacterium avium complex is also important, not just among patients with HIV, but especially in patients with chronic lung disease or cystic fibrosis, and in middle-aged or elderly thin women [115].

C. Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:
Hospital-acquired and ventilator-associated pneumonias (HAP and VAP, respectively) are frequently caused by multidrug-resistant gram-negative bacteria or other bacterial pathogens. Aside from respiratory viruses that may be nosocomially transmitted, viruses and fungi are rare causes of HAP and VAP in the immunocompetent patient. Table 21 lists the organisms most commonly associated with pneumonia in the immunocompetent patient with HAP or VAP.

The 2016 IDSA/ATS guidelines recommend noninvasive sampling of the respiratory tract for both HAP and VAP [116]. In the nonventilated patient, the specimens could include those obtained by spontaneous expectoration, sputum induction, or nasotracheal suction in an uncooperative patient and, in the ventilated patient, endonasotracheal aspirates are preferred [116]. Determining the cause of the pneumonia relies upon initial Gram stain and semi-quantitative cultures of endotracheal aspirates or sputum. A smear lacking inflammatory cells and a culture absent of potential pathogens have a very high negative predictive value. Cultures of endotracheal aspirates, while likely to contain the true pathogen, also consistently grow more mixtures of species of bacteria than specimens obtained by bronchoscopic techniques. This may lead to additional unnecessary antibiotic therapy. Quantitative assessment of invasively obtained samples such as BAL fluid and protected specimen brush specimens is often performed [116]. Quantities of bacterial growth above a threshold are diagnostic of pneumonia and quantities below that threshold are more consistent with colonization. The generally accepted thresholds are as follows: endotracheal aspirates, 106 colony-forming units (CFU)/mL; BAL fluid, 104 CFU/mL; protected specimen brush samples, 103 CFU/mL [116]. Quantitative studies require extensive laboratory work and special procedures that smaller laboratories may not accommodate and are therefore not endorsed by the guidance despite studies that show decreased antibiotic utilization with quantitative cultures [116]. Bronchial washes are not appropriate for routine bacterial culture.

D. Infections of the Pleural Space ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:
An aging population, among other factors, has resulted in an increase in the incidence of pleural infection [117]. The infectious causes of pleural effusions differ between community-acquired and hospital-acquired disease. In a large multicenter study (MIST1) of 454 adult patients with pleural infection to assess streptokinase treatment, the major pathogens recovered in decreasing order of frequency were S. anginosus group, S. aureus, anaerobic bacteria, other streptococci, Enterobacteriaceae, and S. pneumoniae [118]. Among patients with hospital-acquired infection, S. aureus tops the list, with at least half of them being methicillin resistant, followed by Enterobacteriaceae, the streptococci (anginosus group, S. pneumoniae), Enterococcus spp, and anaerobes [118, 119]. Table 22 summarizes the major pathogens. Any significant accumulation of fluid in the pleural space should be sampled by thoracentesis. Specimens should be hand carried immediately to the laboratory or placed into appropriate anaerobic transport media for transport. In some institutions, bedside inoculation into blood culture bottles has become an established practice. This is acceptable and has been shown to increase the sensitivity by 20% [119]. The manufacturer's guidelines should be followed with respect to the volume inoculated and whether supplementation is required to enhance recovery of fastidious pathogens such as S. pneumoniae. If blood culture bottles are used, an additional sample should be sent to the microbiology laboratory for Gram stain and culture of nonbacterial pathogens when indicated. Even when optimum handling occurs, cultures may fail to yield an organism. Laboratory-developed NAATs targeting pneumococcal genes, such as those that encode pneumolysin and autolysin, in fluid from pediatric cases of pleural infection, have been very useful [117].
Fluid should be sent for cell count, pH, protein, glucose, lactate dehydrogenase (LDH), and cholesterol. These values assist with the determination of a transudative or exudative process and in the subsequent management of the syndrome. A recent meta-analysis showed that the best predictors of an exudate were pleural fluid cholesterol level >55 mg/dL and an LDH >200 U/L or the ratio of pleural fluid cholesterol to serum cholesterol >0.3 [120]. Most infections result in an exudate or polymorphonuclear leucocytes (PMNs) (empyema) within the pleural cavity. When tuberculosis or a fungal pathogen is thought to be the likely cause, a pleural biopsy sent for culture and histopathology increases the diagnostic sensitivity. Always notify the laboratory of a suspicion of tuberculosis so that appropriate safety precautions can be employed. The recently published IDSA/ATS/CDC guidelines on the diagnosis of tuberculosis in adults and children "weakly recommends" the measurement of adenosine deaminase (ADA) and free interferon-λ (IFN-λ) in pleural fluid. This endorsement is based upon a sensitivity and specificity of ADA of ≥79% and ≥83%, respectively, as determined by several meta-analyses [121]. The figures for free IFN-λ were ≥89% and ≥97% for sensitivity and specificity, respectively [121]. It should be stressed that the quality of evidence is low and both markers should be used in conjunction with hematologic and chemical parameters and other diagnostic tests such as NAAT, culture, and histology of a pleural biopsy. The performance of ADA in developed countries has been shown to be quite variable and is related to multiple factors including the type of method used, the likelihood of tuberculosis, and "false positive" results in patients with other causes of lymphocytic pleural effusion such as rheumatoid disease, mesothelioma, and histoplasmosis [122].

E. Pulmonary Infections in Cystic Fibrosis ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:
Patients with cystic fibrosis (CF) suffer from chronic lung infections due to disruption of exocrine function that does not allow them to clear microorganisms that enter the distal airways of the lung. The spectrum of organisms associated with disease continues to expand and studies of the microbiome demonstrate the complex synergy between easily cultivatable and noncultivatable organisms. Table 23 lists the most frequently isolated pathogens in this patient population. Early in childhood, infections are caused by organisms frequently seen in the non-CF pediatric population such as S. pneumoniae, H. influenzae, and S. aureus. Of these organisms, methicillin-resistant Staphylococcus aureus (MRSA) has significantly increased in prevalence [123]. At some point, later in childhood or adolescence, P. aeruginosa becomes the most important pathogen involved in chronic lung infection and the concomitant lung destruction that follows. The P. aeruginosa strains adapt to the hypoxic stress of the retained mucoid secretions by converting to a biofilm mode of growth (mucoid colonies). Nosocomial pathogens such as Stenotrophomonas maltophilia, Achromobacter xylosoxidans, and Achromobacter ruhlandii may be acquired during a hospital or clinic visit. Burkholderia cepacia complex is a very important pathogen in these patients. Burkholderia cenocepacia is highly pathogenic and is responsible for rapid decline and death in a subset of patients who acquire the virulent clones. Special microbiological techniques are required to recover and differentiate B. cepacia complex from the mucoid P. aeruginosa strains. Less common gram-negative organisms that appear to be increasing in their frequency of recovery, but whose role in the pathogenesis of CF lung disease is still unclear, include Burkholderia gladioli, Ralstonia spp, Cupriavidus spp, Inquilinus spp, and Pandoraea [124, 125]. The reader is referred to the Parkins and Floto reference for a discussion of pathogens within the CF microbiota [123].
As CF patients have survived into adulthood, opportunistic pathogens such as nontuberculous mycobacteria (NTM) have been isolated with increasing frequency, ranging in prevalence from 6% to up to 30% in patients aged >40 years [123]. The M. avium complex and the Mycobacterium abscessus complex are the most commonly encountered NTM [123]. There is evidence to suggest that both M. abscessus and M. avium complex contribute to lung destruction and should be treated when cultures are repeatedly positive. Mycobacterial culture should be added to the routine cultures obtained from patients >15 years of age who present with exacerbations, as the incidence of Mycobacterium spp is likely underestimated due to failure to routinely assess patients for these organisms [124].

Aspergillus fumigatus is the most common fungus recovered from CF patients, in whom it causes primarily allergic bronchopulmonary disease. Scedosporium apiospermum may cause a similar syndrome. Exophiala dermatitidis has been reported by some centers to cause chronic colonization of the CF airway [124]. Trichosporon mycotoxinivorans is a pathogen that has a propensity to cause disease in patients with CF [125]. Table 23 summarizes the organisms most likely to cause exacerbation of pulmonary symptoms in CF patients [115, 123–127]. While a number of environmental nonfermenting gram-negative bacilli are frequently recovered from the sputum of these patients, their role in CF lung disease is either unknown at this time or unlikely to be of significance. These organisms have not been included in the table. Laboratories should spend resources on those pathogens proven or likely to play a significant role in pulmonary decline in these patients.

F. Pneumonia in the Immunocompromised Host ::: VI. LOWER RESPIRATORY TRACT INFECTIONS:
Advances in cancer treatments, transplantation immunology, and therapies for autoimmune diseases and HIV have expanded the population of severely immunocompromised patients. Pulmonary infections are the most common syndromes contributing to severe morbidity and mortality among these groups of patients.
Virtually any potential pathogen may result in significant illness, and the challenge for both clinicians and microbiologists is to rapidly differentiate infectious from noninfectious causes of pulmonary infiltrates. The likelihood of a specific infection may be affected by recently administered prophylaxis. Table 24 focuses on the major infectious etiologies likely to be of interest in most immunocompromised hosts [128]. Patients are still vulnerable to the usual bacterial and viral causes of CAP and HAP. In addition, fungi, herpesviruses, and protozoa play a more significant role and should be considered.

When rapid and noninvasive tests such as urine or serum antigen tests and rapid viral diagnostics are not revealing, more definitive procedures to sample the lung are required. Several diagnostic procedures can be performed, but usually the patient initially undergoes bronchoscopy with BAL with or without transbronchial biopsy. When an infiltrate is focal, it is important to wedge the scope in the pulmonary segment corresponding to the abnormality on radiographs; otherwise, in diffuse disease, the scope is usually wedged in the right middle lobe or lingula. It is suggested that microbiology laboratories, in collaboration with infectious diseases physicians and pulmonologists, develop an algorithm for processing samples that includes testing for all major categories of pathogens as summarized in the table. Cytologic analysis and/or histopathology are often needed to interpret the significance of positive NAAT or culture for herpesviruses, for example, and to definitively diagnose filamentous fungi. It should be noted, however, that histopathology alone is not sensitive enough to diagnose fungal infections and should be accompanied by immunostain, culture, and, when available, NAATs [128, 129]. In addition, serum and BAL galactomannan and serum 1–3 β-d-glucan tests may be helpful. However, cytology and/or histopathology are quite useful for distinguishing conditions such as pulmonary hemorrhage and rejection from infectious causes of infiltrates. Transthoracic needle aspiration, computed tomography–guided biopsies of pleural-based lesions, and open lung likewise may be considered if less invasive diagnostics are unrevealing.

A. Esophagitis ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Esophagitis is most often caused by noninfectious conditions, such as gastroesophageal reflux disease. Infectious causes are often seen in patients with impaired immunity (Table 25). Fungal microscopy with Calcofluor or potassium hydroxide (KOH) or bacterial examination by Gram stain of esophageal brushings with histopathological examination and viral culture of esophageal biopsies will establish the diagnosis in most cases.

B. Gastritis ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:

Helicobacter pylori is associated with atrophic gastritis, peptic ulcer disease, and gastric cancer. Diagnosis of H. pylori infection is critical as treatment can decrease morbidity. Testing is recommended for all patients with peptic ulcer disease, gastric mucosa–associated lymphoid tissue lymphoma, and early gastric cancer. In some patients with dyspepsia, noninvasive testing is an option [136]. Both invasive and noninvasive tests (Table 26) are available to aid in the diagnosis [137]. Invasive tests such as Gram stain and culture of endoscopy tissue, histopathologic staining, and direct tests for urease require the collection of biopsy samples obtained during endoscopy from patients who have not received antimicrobial agents or proton pump inhibitors in the 2 weeks prior to collection and, as such, pose greater risks to the patient. Culture, although not routinely performed, allows for antimicrobial susceptibility testing. The advantage to the noninvasive assays such as the urea breath test and stool antigen determinations is that patients can avoid endoscopy and gastric biopsy. They are also useful to test for organism eradication after therapy. Collection of specimens for the urea breath test may be performed in the clinic. This assay has a sensitivity of approximately 95%, comparable to the invasive assays. Stool antigen tests have a reported sensitivity of 88%–98%, with sensitivity being higher in adults than in children. The noninvasive assays are also useful to test for organism eradication after therapy, the urea breath test having a somewhat higher sensitivity than stool antigen detection. Serodiagnosis has a lower sensitivity (<90%) and specificity (90%) and is not useful for test of cure after therapy.

Stool Culture ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Stool culture is indicated for detection of invasive bacterial enteric pathogens. When culture methods are employed, most laboratories routinely detect Salmonella, Shigella, and Campylobacter and, more recently, Shiga toxin–producing E. coli in all stools submitted for culture. Salmonella spp can take 24–72 hours to recover and identify to genus alone with the specific serotyping usually performed at the public health laboratory level. It is recommended that tests for the detection of Shiga toxin, or tests to specifically detect Shiga toxin–producing E. coli O157:H7 or other Shiga toxin–producing serotypes, be included as part of the routine test. However, in some settings, these tests may require a specific request. Tests that detect only E. coli O157:H7 will not detect the increasing number of non-O157 isolates being reported and may not detect all E. coli O157:H7 [143]. Screening algorithms that limit testing to bloody stools may

also miss both O157 and non-O157 isolates. Screening of stool for toxin-producing E. coli is recommended for all pediatric patients.
Detection of Vibrio and Yersinia in the United States is usually a special request and requires additional media or incubation conditions. Communication with the laboratory is necessary. Laboratory reports should indicate which of the enteric pathogens would be detected. Laboratories are encouraged to provide enteric pathogen isolates to their public health laboratory and/or the CDC for pulsed-field gel analysis or whole-genomic sequencing for national surveillance purposes. Culture methods must be used for test of cure.
Selective use of multiplex NAATs for stool pathogens is very sensitive and, when positive for reportable agents, should either be cultured to recover the isolate or the stool provided to public health laboratories to culture, for epidemiologic follow-up.

Culture-Independent Methods ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Culture independent methods are becoming increasingly available. Nucleic acid amplification assays vary from singleplex to highly multiplexed assays. It is imperative to communicate with the laboratory to determine what organisms are detected. Culture independent methods can detect pathogens in as little as 1–5 hours compared to the 24–96 hours often required for culture. These assays are reported to be more sensitive than culture and have resulted in much higher rates of detection [144]. Highly multiplexed assays allow for the detection of mixed infections, where the importance of each pathogen is unclear, and they may allow for the detection of pathogens, such as enteroaggregative E. coli or sapovirus, where the indication for therapy is unclear. Culture-independent methods should not be used as test of cure as they will detect both viable and nonviable organisms.

Clostridium botulinum ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Botulism is an intoxication in which a protein exotoxin, botulinum toxin, produced by Clostridium botulinum causes a life-threatening flaccid paralysis. Diagnosis, while not usually confirmed by the hospital microbiology laboratory, is made by clinical criteria, allowing prompt initiation of essential antitoxin therapy. The microbiologic diagnosis is dependent upon detection of botulinum toxin in serum (in patients with wound, infant, and foodborne disease), stool (in patients with infant and foodborne disease), and gastric contents/vomitus (in patients with foodborne disease). Toxin detection is performed in many public health laboratories and at the CDC. Culture can be performed on both feces and wounds, but the yield is low and most laboratories lack the necessary expertise to isolate and identify this organism [145].

Clostridium difficile ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Numerous methods have been employed for the laboratory diagnosis of infection caused by Clostridium difficile. Toxigenic culture is probably the most sensitive and specific of the assays for the detection of C. difficile, although detection of a toxigenic organism is not, in itself, specific for infection. It is slow and labor intensive and not routinely performed in the community hospital setting. Compared to toxigenic culture, the cytotoxin assay has a sensitivity of 85%–90%. The cytotoxin assay requires 24–48 hours and is also labor intensive. Thus, toxin detection by either enzyme immunoassay (EIA) or immunochromatographic methods are widely used in clinical practice. These assays have reported sensitivity of 70%–85% but are significantly faster with results available in <2 hours. Utilization of an assay that detects both toxin A and toxin B improves the sensitivity. Glutamate dehydrogenase (GDH) antigen assays are sensitive but have poor specificity. NAATs for the detection of C. difficile have reported sensitivity of 93%–100%. To reduce turnaround time, reduce costs, and improve accuracy of C. difficile–associated disease, some laboratories employ an algorithm that utilizes GDH as a rapid screening test, followed by (or simultaneous to, as part of the same test platform), EIA for toxin A and B detection with or without cytotoxin testing or NAAT to arbitrate discrepant GDH and EIA toxin results. These algorithms allow for both the rapid reporting of most negative specimens and the sensitivity of cytotoxin testing or NAAT, but could result in delays depending on the laboratory testing algorithm employed [146–148]. NAAT detects viable and nonviable organisms. To decrease the identification of colonized patients, some laboratories are performing both NAAT tests and tests to detect toxin production. Diarrheal stool specimens (not formed stools or rectal swabs) are required for the diagnosis of C. difficile disease (not colonization). The specimen should be loose enough to take

the shape of the container. Formed stools should be appropriately rejected by the laboratory but with the proviso that formed stools from patients with ileus, or potential toxic megacolon, as noted by the physician, should be tested. When testing is limited to patients not receiving laxatives and with unexplained and new-onset diarrhea (≥3 unformed stools in 24 hours), NAAT alone, or toxin EIA as part of a multistep algorithm (GDH plus toxin, GDH plus toxin arbitrated by NAAT, or NAAT plus toxin) are the recommended test options. When there are no institutionally agreed upon limiting criteria for stool submission, toxin EIA, as part of a multistep algorithm as defined above, is recommended, not NAAT testing alone. Repeat testing of patients previously positive as a “test of cure” is not appropriate. Repeat testing of patients negative by NAATs should not be performed for at least 6 days [148, 149].

Because of the presence of asymptomatic carriage, routine testing should not be performed in children <2 years of age, particularly in those <1 year (infants) [150]. Toxigenic C. difficile colonizes nearly 50% of infants in the first year of life, with asymptomatic rates at around 2 years of age approaching those of healthy adults. The presence of diarrhea is difficult to assess in this age group as loose or unformed stool can be difficult to discriminate. However, there are data to suggest that C. difficile may be the cause of disease in some infants. For children <2 years of age, testing for other causes should be pursued first, with C. difficile testing being performed only if there is no alternative cause and the symptoms are severe or the clinical presentation is consistent with C. difficile infection [151].

Since 2000, an increase in C. difficile–associated disease with increased morbidity and mortality has been reported in the United States, Canada, and the United Kingdom. The epidemic strain is toxinotype III, North American pulsed-field gel electrophoresis (PFGE) type 1 (NAP1), and PCR ribotype 027 (NAP1/027). It carries the binary toxin genes, cdtA and cdtB, and an 18-bp deletion in tcdC. It produces both toxin A and toxin B [152]. A commercially available FDA-cleared NAAT for binary toxin and the tcdC deletion genes identifies this strain for epidemiological purposes. The severity of disease is believed to be due to toxin hyperproduction [153]. The association of binary toxin with disease severity is controversial.

Parasites ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:

The number of specimens to be submitted for parasitologic examination may be a controversial subject [154, 155]. Historically, when using conventional microscopic procedures, it was recommended that 3 specimens collected over a 7- to 10-day period be submitted for ova and parasite (O&P) examination. Options for cost-effective testing today include examination of a second specimen only when the first is negative and the patient remains symptomatic, with a third specimen being submitted only if the patient continues to be O&P negative and symptomatic. Targeted use of immunoassay testing or NAAT for the most common parasites based on geography, patient demographics, and physician request can also be used as a screen, with only negative patients with continued symptoms or patients with specific risk factors requiring full O&P examination. Immunoassays for Giardia are sensitive enough that only a single specimen may be needed. No data are available on the number of specimens required to rule out infection when NAAT is performed.

The specimen preservative to be used, often supplied by the laboratory, depends on the need to perform immunoassay procedures or special stains or NAAT on the specimens and the manufacturer’s recommendations for specimen fixative. It is imperative that the laboratory be consulted to assure proper transport conditions are utilized. Polyvinyl alcohol is the gold standard for microscopic examination; however, due to the presence of mercuric chloride, modifications that do not employ mercury have been developed. None of these modified preservatives allow stains to provide the same level of microscopic detail, although with experience, they are acceptable alternatives.

In routine procedures, pathogenic Entamoeba histolytica cannot be differentiated from nonpathogenic Entamoeba dispar using morphologic criteria, so the laboratory report may indicate E. histolytica/dispar [156]. Only an immunoassay or NAAT can differentiate these organisms.

Viruses ::: C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:

Viral causes of gastroenteritis are often of short duration and self-limited. Viral shedding may persist after resolution of symptoms. Although included as part of some multiplex NAAT, testing is not routinely performed except in immunocompromised patients, infection control purposes, or

outbreak investigations. In immunocompromised hosts, laboratory testing for CMV should be considered, using a quantitative NAAT performed on plasma. Of note, a negative NAAT does not rule out the possibility of CMV disease, and repeat testing may be required.

Proctitis ::: VII. INFECTIONS OF THE GASTROINTESTINAL TRACT:
Proctitis is most commonly due to sexually transmitted agents, a result of anal–genital contact, although abscesses or perirectal wound infections may present with similar symptoms. One sample is usually sufficient for diagnosis (Table 28).

A. Spontaneous Bacterial Peritonitis and Ascites ::: VIII. INTRA-ABDOMINAL INFECTIONS:
In cases of spontaneous bacterial peritonitis (SBP), the source of the invading organism(s) is unknown and the syndrome can also be seen in patients with preexisting risk factors such as cirrhosis with ascites [157, 158]. SBP is an ascitic fluid infection without an evident intra-abdominal focus, tends to be monomicrobic, and is usually caused by aerobic organisms from the intestinal tract; therefore, anaerobic cultures are less valuable. Sufficient fluid (eg, 10–50 mL if available) should be obtained to allow for concentration by centrifugation and a cytospin Gram stain evaluation. At a minimum, at least 10 mL of peritoneal fluid (not swabs of the fluid) should be collected aseptically and transported to the laboratory prior to the administration of antimicrobial agents. Additional laboratory testing should include fluid analysis for protein, cell count and differential, lactate concentration, and pH along with 2–3 sets of blood cultures for the identification of concomitant bacteremia (Table 29). Alternatively, because SBP and infections of ascites fluid tend to be monomicrobic, an aerobic blood culture bottle can be inoculated with fluid (volume dependent upon blood culture system) if the presence of a single organism is reasonably certain. A Gram stain may be used prior to broth inoculation to evaluate the morphology of any organism(s) present in the specimen. Since the differentiation between SBP and secondary peritonitis may be uncertain, it may be beneficial to submit peritoneal fluid in a sterile container for conventional culture and stain as well as to inoculate blood culture bottles at the bedside with the fluid. Mass spectrometry, sequencing, and 16S PCR can be used to identify isolates present in these specimens if these techniques are available to the laboratory. In the next few years, next-generation sequencing will be able to analyze such specimens to determine the total microbial load by species. If >1 morphologic type is noted in the Gram stain, a broth should not be inoculated. The caveat for use of blood culture bottles with fluid other than blood is that not all systems have been evaluated for this purpose. Furthermore, broth cultures do not accurately reflect the bacterial burden or the variety of organisms at the time the specimen is obtained, and the presence of a true pathogen may be obscured by the overgrowth of a more rapidly growing organism.
Negative culture results in the presence of other indicators of infection should prompt an evaluation for fastidious or slowly growing organisms such as Mycobacterium spp, fungi, Chlamydia trachomatis, or Neisseria gonorrhoeae.

B. Secondary Peritonitis ::: VIII. INTRA-ABDOMINAL INFECTIONS:
The diagnosis of secondary peritonitis is dependent upon identifying a source for invading microorganisms—usually genitourinary or gastrointestinal flora [158, 159]. There are numerous causes of secondary peritonitis including iatrogenic or accidental trauma, perforated appendix or diverticuli, typhlitis, or intra-abdominal abscess. Complications from bariatric surgery may also cause secondary peritonitis. Unlike SBP, however, secondary peritonitis tends to be polymicrobic and may include anaerobic flora. Organisms such as S. aureus, N. gonorrhoeae, and Mycobacterium spp are unusual in this setting. Common etiologies include aerobic and anaerobic gram-negative rods (Bacteroides spp, E. coli, Klebsiella spp) and gram-positive flora (Clostridium spp, Enterococcus spp, Bifidobacterium spp, Peptostreptococcus spp). Infectious complications following bariatric surgery are frequently due to gram-positive cocci and yeast (Candida spp). Since many obese patients have had prior exposure to antibiotics, multidrug-resistant organisms are of concern [160, 161]. If typhlitis is suspected, C. difficile toxin testing, stool cultures for enteric pathogens, and blood cultures should be requested. Additionally, Clostridium septicum should be considered in neutropenic enterocolitis.
Peritoneal fluid should be sent to the laboratory in an anaerobic transport system for Gram stain and aerobic and anaerobic bacterial cultures. Inoculation of blood culture bottles alone with peritoneal fluid is not appropriate in this setting, as competitive bacterial growth in broth cultures could mask the recovery of clinically important pathogens (Table 29). Because CMV is a possible cause of secondary peritonitis, the microbiology laboratory should be contacted to arrange for

special processing if CMV is of concern. The microbiology laboratory should also be contacted if N. gonorrhoeae is of concern as special processing or NAAT (this specimen type has no FDA-cleared commercial platform for testing) will be necessary.
Because of the polymicrobic nature of secondary peritonitis, clinicians and other healthcare providers should not expect or request identification and susceptibility testing of all organisms isolated. Rather, the laboratory should provide a general description of the culture results (eg, mixed aerobic and anaerobic intestinal flora) and selective identification of certain organisms such as MRSA, β-hemolytic Streptococcus spp, multidrug-resistant gram-negative bacilli, and vancomycin-resistant enterococci (VRE), etc) to guide empiric antimicrobial therapy [157, 158, 162]. Patients who do not respond to conventional therapy should have additional specimens collected to examine for resistant organisms or for the presence of intra-abdominal abscesses.

C. Tertiary Peritonitis ::: VIII. INTRA-ABDOMINAL INFECTIONS:
This entity refers to persistent or recurrent peritonitis following unsuccessful treatment of secondary peritonitis. Tertiary peritonitis might also indicate the presence of an intra-abdominal abscess or organisms that are refractory to broad-spectrum antimicrobial therapy such as VRE, Candida spp, Pseudomonas aeruginosa, or biofilm-producing bacteria such as coagulase-negative Staphylococcus spp. Fluid cultures from cases of tertiary peritonitis are commonly negative for bacteria [157]. In any case, cultures appropriate for spontaneous or secondary peritonitis may be helpful (Table 30). The possibility of infection caused by unusual or slowly growing organisms such as filamentous fungi and Mycobacterium spp should be entertained if bacterial cultures are negative for growth. If culture results in growth of Mycobacterium spp, it may represent disseminated disease. However, AFB and parasitic studies would only rarely be considered.

D. Peritoneal Dialysis–Associated Peritonitis ::: VIII. INTRA-ABDOMINAL INFECTIONS:
The evaluation of dialysis fluid from patients with suspected peritoneal dialysis–associated peritonitis (PDAP) is essentially identical to that used for SBP. Infections tend to be monomicrobic and rarely anaerobic. In the case of PDAP, however, the list of likely suspect organisms is quite different from SBP. Gram-positive bacteria (predominantly Staphylococcus spp and, to a lesser extent, Streptococcus and Corynebacterium spp) account for >60% of cultured microorganisms. Gram-negative bacteria (mostly E. coli, Klebsiella, and Enterobacter spp) represent <30% of positive cultures while anaerobes comprise <3% of isolates [158, 163, 164]. Fungi, especially Candida spp, contribute to the same number of identified infections as anaerobes [165]. Cultures can remain negative in >20% of all cases of PDAP [163]. Again, 10–50 mL of dialysate should be collected for concentration and culture, cytospin Gram stain evaluation, analysis for protein, and cell count and differential (Table 30). Blood cultures are rarely positive in cases of PDAP [158]. Direct inoculation of dialysate or a concentrated dialysate into an aerobic blood culture bottle for automated detection has proven to be as effective as direct plating of centrifuged fluid [164, 165]. Consult directly with the microbiology laboratory when primary cultures of fluid are negative and additional cultures for slowly growing or highly fastidious organisms such as Mycobacterium, Nocardia, and filamentous fungi should be pursued. If Nocardia is of concern, primary culture plates require prolonged incubation or culture on fungal media or buffered charcoal yeast extract agar.

E. Space-Occupying Lesions of the Liver ::: VIII. INTRA-ABDOMINAL INFECTIONS:
The primary diagnostic dilemma for cases of space-occupying lesions of the liver is distinguishing those caused by parasites (E. histolytica and Echinococcus) from pyogenic abscesses caused by bacteria or fungi. The location, size, and number of liver abscesses is often not helpful for differentiation purposes as the majority are in the right lobe and can be seen in single or multiple loci [166–168]. In regions where E. histolytica disease is endemic, the use of serology or serum antigen detection tests can be helpful to exclude amebic abscess [169], whereas examination of stool for cysts and trophozoites is generally not (Table 30). Liver abscess aspirates can be tested for the presence of E. histolytica antigen as well as submitted for direct microscopic evaluation for parasites. When amebic disease is unlikely, the abscess should be aspirated and the contents submitted in anaerobic transport for aerobic and anaerobic bacterial cultures. Commonly recovered isolates include Klebsiella spp, E. coli, and other Enterobacteriaceae, Pseudomonas spp, Streptococcus spp including Streptococcus anginosus group spp, Enterococcus spp, viridans group streptococci, S. aureus, Bacteroides spp, Fusobacterium spp (especially with Lemierre syndrome), Clostridium spp, and, rarely, Candida spp [166–168]. Aerobic and anaerobic

bacterial culture should be requested (Table 30). Blood cultures can also be helpful in establishing an etiology if collected prior to the institution of antimicrobial therapy [167, 168]. Occasionally, patients with primary genital infections due to N. gonorrhoeae or C. trachomatis can have extension of the disease to involve the liver capsule or adjacent peritoneum (Fitz-Hugh–Curtis syndrome).

F. Infections of the Biliary Tree ::: VIII. INTRA-ABDOMINAL INFECTIONS:
Not unexpectedly, bacteria commonly associated with biliary tract infections (primarily cholecystitis and cholangitis) are the same organisms recovered from cases of pyogenic liver abscess (see above and Table 29). Parasitic causes include Ascaris and Clonorchis spp or any parasite that can inhabit the biliary tree leading to obstruction [166]. At a minimum, cultures for aerobic bacteria (anaerobes if the aspirate is collected appropriately) and Gram stain should be requested. When signs of sepsis and peritonitis are present, blood and peritoneal cultures should be obtained as well.
For patients with HIV infection, the list of potential agents and subsequent microbiology evaluations needs to be expanded to include Cryptosporidium, microsporidia, Cystoisospora (Isospora) belli, CMV, and M. avium complex [166]. As the identification of these organisms requires special processing, it is important to communicate with the laboratory to determine test availability either on-site or at a reference laboratory.

G. Splenic Abscess ::: VIII. INTRA-ABDOMINAL INFECTIONS:
Most cases of splenic abscess are the result of metastatic or contiguous infectious processes, trauma, splenic infarction, or immunosuppression [169]. Infection is most likely aerobic and monomicrobic with Staphylococcus spp, Streptococcus spp, Enterococcus spp, Salmonella spp, and E. coli commonly isolated. Anaerobic bacteria have been recovered in 5%–17% of culture-positive cases [170]. Aspirates should be processed in a similar manner as pyogenic liver abscesses including aerobic and anaerobic culture, Gram stain, and concomitantly collected blood culture sets (Table 30). Unusual causes of splenic abscess include Bartonella spp, Brucella melitensis, Streptobacillus moniliformis, Nocardia spp, and Burkholderia pseudomallei (uncommon outside of Southeast Asia or without suggestive travel history) [171]. The laboratory should be notified if B. melitensis or B. pseudomallei is possible due to the need for increased biosafety/security precautions since they are potential bioterrorism agents. As in biliary disease, the spectrum of organisms to be considered needs to be expanded to include Mycobacterium spp, fungi (including Pneumocystis jirovecii), and parasites for immunocompromised patients [171].

H. Secondary Pancreatic Infection ::: VIII. INTRA-ABDOMINAL INFECTIONS:
Most cases of acute or chronic pancreatitis are produced by obstruction, autoimmunity, or alcohol ingestion [172, 173]. Necrotic pancreatic tissue generated by one of these processes can serve as a nidus for infection [172, 173]. Infectious agents associated with acute pancreatitis are numerous and diverse; however, superinfection of the pancreas is most often caused by gastrointestinal flora such as E. coli, Klebsiella spp, and other members of the Enterobacteriaceae, Enterococcus spp, Staphylococcus spp, Streptococcus spp, and Candida spp. Necrotic tissue or pancreatic aspirates should be sent for aerobic bacterial culture and Gram stain and accompanied by 2–3 sets of blood cultures (Table 30). Antimicrobial susceptibility results from isolated organisms can be used to direct therapy to reduce the likelihood of pancreatic sepsis, further extension of infection to contiguous organs, and mortality. Sterile cultures of necrotic pancreatic tissue are not unusual but may trigger consideration of an expanded search for fastidious or slowly growing organisms, parasites, or viruses.

A. Osteomyelitis ::: IX. BONE AND JOINT INFECTIONS:
Osteomyelitis can occur following hematogenous spread, after a contaminated open fracture, or in those with diabetes mellitus or vascular insufficiency. Vertebral osteomyelitis/spondylodiscitis will be separately considered. Osteomyelitis is typically suspected on clinical grounds, with confirmation involving imaging and microbiologic and histopathologic tests. The peripheral white blood cell count may be elevated, and the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are often elevated. Establishing an etiologic diagnosis, which is important for directing appropriate clinical management since this varies by microorganism type and associated antimicrobial susceptibility, nearly always requires obtaining bone for microbiologic evaluation. This can be accomplished by imaging-guided or surgical sampling. As much specimen as

possible should be submitted to the laboratory; specimens may include pieces of intact bone, shavings, scrapings, and/or excised or aspirated necrotic material (Table 31). Swabs are not recommended. Cultures of sinus tracts are generally not recommended because recovered organisms usually do not correlate with those found in deep cultures, although S. aureus shows modest correlation. Hematogenous osteomyelitis is usually monobacterial, whereas that resulting from contiguous infection is often polymicrobial. Acute hematogenous osteomyelitis of long bones mainly occurs in prepubertal children, but can occur in the elderly, injection drug users, and those with indwelling central venous catheters. In prepubertal children, the most common microorganisms involved are S. aureus and S. pneumoniae; Kingella kingae is common in children <4 years of age [174]. Osteomyelitis in neonates, especially in those with indwelling central venous catheters, typically results from hematogenous spread; commonly involved organisms include Streptococcus agalactiae and aerobic gram-negative bacteria, especially E. coli. Candida spp and P. aeruginosa are more commonly encountered in injection drug users and those with indwelling central venous catheters. In children, the diagnosis is often made based on clinical and imaging findings in the context of positive blood cultures. NAATs are particularly useful for diagnosing K. kingae bone and joint infection in children <4 years of age. In adults, imaging-guided aspiration or open biopsy is typically necessary.

In osteomyelitis occurring after a contaminated open fracture, the organisms listed above may be found, with enterococci, fungi, and NTM alternatively being involved; microorganisms may derive from patient skin, contaminated soil, and/or the healthcare environment.

In patients with diabetes, osteomyelitis typically involves the foot as a complication of a chronic foot ulcer; a positive probe-to-bone test is associated with osteomyelitis. Specimens for bone culture (aerobic and anaerobic) and histology can be obtained by open debridement, needle puncture, or transcutaneous biopsy. Readers are referred to a guideline that provides greater detail on the diagnosis of diabetic foot infections [175].

Vertebral osteomyelitis/disc space infection/spondylodiscitis is often hematogenous in origin (eg, from skin and soft tissue, urinary tract, intravascular catheter, pulmonary infection sites), but can occur postoperatively or following a procedure. Staphylococcus aureus and coagulase-negative staphylococci are most commonly involved, followed by gram-negative aerobes, streptococci, Candida spp, and, in patients with relevant risk factors, Mycobacterium tuberculosis (and occasionally NTM) and Brucella spp. Two sets of aerobic and anaerobic bacterial/candidal blood cultures and ESR and CRP should be obtained; in addition, Brucella blood cultures and serologic tests should be obtained in those in areas endemic for brucellosis, fungal blood cultures in those with relevant epidemiologic or host risk factors, and, as with other types of osteomyelitis, a purified protein derivative test or interferon-γ release assay may be considered in those at risk for tuberculosis (acknowledging a risk of both false-positive and false-negative results). Patients suspected of having native vertebral osteomyelitis based on clinical, laboratory, and imaging studies, with S. aureus, Staphylococcus lugdunensis, or Brucella bloodstream infection or, in an endemic setting, a positive Brucella serology, do not need further testing. For all others, imaging-guided aspiration/biopsy of a disc space or vertebral endplate is recommended, with the specimens submitted for Gram stain and aerobic and anaerobic culture and, if adequate tissue can be obtained, histopathology. If results are negative or inconclusive (eg, Corynebacterium spp is isolated), a second imaging-guided aspiration biopsy, percutaneous endoscopic discectomy and drainage procedure, or open excisional biopsy should be considered to collect additional specimens for repeat and additional testing. Readers are referred to a guideline that provides greater detail on the diagnosis of native vertebral osteomyelitis in adults [176].

B. Infections of Native Joints and Bursitis ::: IX. BONE AND JOINT INFECTIONS:

Joints can be hematogenously seeded by bacteria, or seeded by direct inoculation or from a contiguous focus, with the majority of infections being monoarticular. Staphylococcus aureus and Streptococcus spp are common causes of septic arthritis of native joints, followed by gram-negative bacilli, which mainly cause septic arthritis in neonates, the elderly, injection drug users, and the immunocompromised. Kingella kingae is the most common etiology of bacterial joint infection in children <4 years of age. Gonococcal arthritis is rare. Viruses, including parvovirus B19, Chikungunya virus, and rubella, among others, may be associated with arthritis (Table 32). Subacute or chronic infectious arthritis may be caused by M. tuberculosis and NTM, Borrelia burgdorferi, Candida spp, Blastomyces sp, Coccidioides immitis/posadasii, Histoplasma spp, Sporothrix sp, Cryptococcus neoformans/gattii, and Aspergillus spp, among others. Septic bursitis, which usually involves the prepatellar, olecranon, or trochanteric bursae, is usually caused by S. aureus.

Although peripheral-blood white cell count, ESR, and CRP are often elevated, they are nonspecific. Arthrocentesis of a septic joint usually reveals purulent, low-viscosity synovial fluid with an elevated neutrophil count. Traditionally, a synovial fluid leukocyte count >50000 cells/μL was considered to suggest septic arthritis; however, lower counts do not exclude the diagnosis. Ideally, synovial fluid should be submitted for Gram stain, and cultured in aerobic and anaerobic blood culture bottles. If synovial fluid studies are negative, biopsy of the synovium may be required for Gram stain, aerobic and anaerobic cultures, histopathologic evaluation, and possibly fungal and mycobacterial stains and cultures. Concomitant or secondary bacteremia or fungemia occurs sporadically in patients with septic arthritis; thus, blood cultures collected during febrile episodes are recommended.

C. Prosthetic Joint Infection ::: IX. BONE AND JOINT INFECTIONS:
A special category of bone and joint infection exists for prosthetic joint infection (PJI), which may involve knee, hip, shoulder, elbow, or other prostheses [177]. Staphylococci, including not just S. aureus, but also the coagulase-negative staphylococci, especially Staphylococcus epidermidis, are particularly common causes, but many other organisms, including streptococci, enterococci, aerobic gram-negative bacilli, anaerobic bacteria (eg, Cutibacterium acnes, Finegoldia magna), and fungi, can be involved (Table 33). Cutibacterium acnes is particularly common in shoulder arthroplasty infection.
The diagnosis of PJI is ideally made preoperatively, but if this is not possible, diagnosis and, if present, definition of the infecting organism(s) should be pursued at the time of revision or resection arthroplasty. Readers are referred to a guideline that provides detail on the diagnosis of PJI [178]. Preoperatively, ESR and CRP are recommended, as is arthrocentesis for synovial fluid cell count, differential, and culture, ideally in aerobic and anaerobic blood culture bottles. Criteria for the interpretation of synovial fluid cell count and differential in the presence of a prosthetic joint differ from those in native joints. Intraoperative frozen section analysis is a reliable diagnostic test. For tissue culture, multiple specimens should be submitted for aerobic and anaerobic cultures, 4 if using conventional plate and broth cultures, and 3 if culturing tissues in aerobic and anaerobic blood culture bottles [179]. Tissue can be processed in a number of ways, including crushing, stomaching, and bead mill processing using glass beads [180]. Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures that yield the same organism is considered definitive evidence of PJI. Notably, single positive tissue or synovial fluid cultures, especially for organisms that may be contaminants (eg, coagulase-negative staphylococci, C. acnes), should not be considered evidence of definite PJI. Gram stains are not recommended. Isolation of C. acnes may require culture incubation times as long as 14 days. The pathogenesis of PJI relates to the presence of microorganisms in biofilms on the implant surface. Therefore, if the arthroplasty is resected, the implant components may be vortexed and sonicated and the resultant sonication fluid semi-quantitatively cultured [181]. Since fungi and mycobacteria are extremely rare in this setting, they should not be routinely sought.

A. Urinary Tract Infection/Pyelonephritis ::: X. URINARY TRACT INFECTIONS:
The IDSA guidelines for diagnosis and treatment of UTIs are published [182, 183] as are American Society for Microbiology recommendations [184]. These provide diagnostic recommendations that are similar to those presented here (Table 34). The differentiation of cystitis and pyelonephritis requires clinical information and physical findings as well as laboratory information, and from the laboratory perspective the spectrum of pathogens is similar for the 2 syndromes [185]. Culturing only urines that have tested positive for pyuria, either with a dipstick test for leukocyte esterase or other indicators of PMNs, may increase the likelihood of a positive culture, but occasionally samples yielding positive screening tests yield negative culture results and vice versa [186]. The Gram stain is not the appropriate method to detect PMNs in urine, but it can be ordered as an option for detection of high numbers of gram-negative rods when a patient is suspected of suffering from urosepsis. Because urine is so easily contaminated with commensal flora, specimens for culture of bacterial urinary tract pathogens should be collected with attention to minimizing contamination from the perineal and superficial mucosal microbiota [187]. Although some literature suggests that traditional skin cleansing in preparation for the collection of midstream or “clean catch” specimens is not of benefit, many laboratories find that such specimens obtained without skin cleansing routinely contain mixed flora and, if not stored properly and transported within 1 hour to the laboratory, yield high numbers of one or more potential pathogens on culture. Determining the true etiologic agent in such cultures is difficult, so skin cleansing is still recommended. The use of urine transport media in vacuum-fill tubes or

refrigeration immediately after collection may decrease the proliferation of small numbers of contaminating organisms and increase the numbers of interpretable results. Straight or “in-and-out” catheterization of a properly prepared patient usually provides a less contaminated specimen. If mixed enteric bacteria in high numbers are recovered from a second, well-collected, straight-catheterized sample from the same patient, a enteric-urinary fistula should be considered. Laboratory actions should be based on decisions arrived at by dialogue between clinician and laboratory.

Specimens from urinary catheters in place for more than a few hours frequently contain colonizing flora due to rapid biofilm formation on the catheter surface, which may not represent infection. Culture from indwelling catheters is therefore strongly discouraged, but if required, the specimen must be taken from the sampling port of a newly inserted device. Cultures of Foley catheter tips are of no clinical value and will be rejected. Collection of specimens from urinary diversions such as ileal loops is also discouraged because of the propensity of these locations to be chronically colonized. Chronic nephrostomy collections and bagged urine collections are also of questionable value. Multiple organisms or coagulase-negative staphylococci may be recovered in patients with urinary stents, and may be pathogenic. It is important that urologists and nephrologists who care for patients with complicated infections discuss any special needs or requests with the microbiology director or supervisor. Specimens from these patients may contain a mixed flora and if specific interpretive criteria are documented for these specimen types, the laboratory must be aware of the documentation and the special interpretive standards. Laboratories routinely provide antimicrobial susceptibility tests on potential pathogens in significant numbers. Specimens obtained by more invasive means, such as cystoscope or suprapubic aspirations, should be clearly identified and the workup discussed in advance with the laboratory, especially if the clinician is interested in recovery of bacteria in concentrations <1000 CFU per milliliter. Identification of a single potential pathogen in numbers as low as 200 CFU/mL may be significant, such as in acute urethral syndrome, but requests for culture results reports of <10000 CFU/mL should be coordinated with the laboratory so that an appropriate volume of urine can be processed.

While not without some exceptions, in febrile infants and young children (2–24 months) an abnormal urinalysis and a colony count of >50000 CFU/mL of a single organism obtained by either a suprapubic aspirate or catheterization is considered diagnostic [188]. More recent evidence would suggest that ≥104 CFU/mL and a reliable detection of pyuria would pick up an additional significant proportion of children with true UTI [189].

Recovery of yeast, usually Candida spp, even in high CFU/mL, is not infrequent from patients who do not actually have yeast UTI, thus interpretation of cultures yielding yeast is not as standardized as that for bacterial pathogens. Yeast in urine may rarely indicate systemic infection, for which additional tests must be conducted for confirmation (eg, blood cultures and β-glucan levels).

Recovery of Mycobacterium tuberculosis is best accomplished with first-void morning specimens of >20 mL, and requires a specific request to the laboratory so that appropriate processing and media are employed. Detection of adenovirus in cases of cystitis is usually done by NAAT. This testing is typically available at tertiary academic centers or reference laboratories. Polyoma BK virus nephropathy is best diagnosed by quantitative molecular determination of circulating virus in blood rather than detection of virus in urine. Such tests are usually performed in tertiary medical centers or reference laboratories.

B. Prostatitis ::: X. URINARY TRACT INFECTIONS:

Acute bacterial prostatitis is defined by clinical signs and physical findings combined with positive urine or prostate secretion cultures yielding usual urinary tract pathogens [190–192]. The diagnosis of chronic prostatitis is much more problematic, and the percentage of cases in which a positive culture is obtained is much lower [193]. The traditional Meares-Stamey 4-glass specimen obtained by collecting the first 10-mL void, a midstream specimen, expressed prostate secretions (EPSs) and a 10-mL post–prostate massage urine is positive if there is a 10-fold higher bacterial count in the EPS than the midstream urine. A 2-specimen variant, involving only the midstream and the EPS specimens, is also used. A positive test is infrequent, and chronic pelvic pain syndrome is not frequently caused by a culturable infectious agent. It should be remembered that prostatic massage in a patient with acute bacterial prostatitis may precipitate bacteremia and/or shock. Table 35 summarizes the approach to laboratory diagnosis of prostatitis.

C. Epididymitis and Orchitis ::: X. URINARY TRACT INFECTIONS:

Epididymitis in men <35 years of age is most frequently associated with the sexually transmitted pathogens Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC). NAATs are the most sensitive and a rapid diagnostic procedure for these agents, and each commercially available system has its own collection kit. Culture of GC is recommended when antibiotic resistance is a concern, and special media are required for antimicrobial susceptibility testing, which may be referred to a public health laboratory. In men >35 years of age, gram-negative and gram-positive pathogens similar to the organisms causing UTI and prostatitis may cause invasive infections of the epididymis and testis. Surgically obtained tissue may be cultured for bacterial pathogens, and antimicrobial susceptibility testing will be performed. Fungal and mycobacterial disease are both uncommon, and laboratory diagnosis requires communication from the clinician to the laboratory to ensure proper medium selection and processing, particularly if tissue is to be cultured for these organisms.
Bacterial orchitis may be caused by both gram-negative and gram-positive pathogens, frequently by extension from a contiguous infection of the epididymis. Viral orchitis is most frequently ascribed to mumps virus. The diagnosis is made by IgM serology for mumps antibodies, or by acute and convalescent IgG serology. Other viral causes of epididymo-orchitis are Coxsackie virus, rubella virus, Epstein-Barr virus, and varicella zoster virus (VZV). Systemic fungal diseases can involve the epididymis or testis, including blastomycosis, histoplasmosis, and coccidioidomycosis. Mycobacterium tuberculosis may also involve these sites [194]. Table 36 summarizes the approaches to specimen management for cases of epididymitis and orchitis.

A. Genital Lesions ::: XI. GENITAL INFECTIONS:
Genital lesions may have multiple simultaneous infectious etiologies that make them a challenge to diagnose and treat properly. Guidelines from the CDC recommend that all patients presenting with a genital lesion should be evaluated with a serological test for syphilis, as well as diagnostic tests for genital herpes and for Haemophilus ducreyi where chancroid is prevalent. Because many of the genital lesions exhibit inflammatory epithelium that enhances the transmission of HIV, serologic screening for HIV infection is recommended in these patients as well [195]. Table 37 shows the diagnostic tests for identifying the etiology of the most common genital lesions.
For suspected cases of HSV genital lesions, viral culture, DFA and/or NAATs are commonly used for diagnosis. As methods for specific testing for vesicles varies among laboratories, consultation with the laboratory before specimen collection is appropriate. While many NAATs are FDA-cleared and the preferred diagnostic because they provide typing and are the most sensitive, especially where suboptimal collection or nonulcerative or vesicular lesions may be present, there may be limitations as to specimen source able to be tested and/or patient age depending on the NAAT used. Culture is more likely to be positive in patients that have vesicular vs ulcerative lesions, specimens obtained from a first episodic lesion vs a recurrent lesion, and specimens from immunosuppressed patients rather than immunocompetent. DFA allows assessment of an adequate specimen and can be a rapid test if performed on-site; isolates should be typed to determine if they are HSV-1 or HSV-2 since 12-month recurrence rates are more common with HSV-2 (90%) than HSV-1 (55%). Serology cannot distinguish between HSV-1 and HSV-2 unless a type-specific glycoprotein G–based assay is requested [195, 197]
Point-of-care tests or tests that can be signed up for online and are antibody-serologic tests should not be used in patient populations with a low likelihood of HSV infection (no symptoms, no high-risk history) because a low index value positive is not specific and often yields false-positive results. In one study where HSV-2–positive indexes were reviewed, increasing the cutoff index value yielded better specificity. Similarly, early stages of infection result in false-negative results. In children presenting with genital lesions, providers should not assume HSV as the only etiology and should consider potential atypical presentation of VZV. DFA and NAATs are best for detection of VZV, as culture is less sensitive. No NAAT FDA-cleared assays are available at the time of this writing, although some laboratories may offer a laboratory-developed test (LDT). Pregnant patients with a history of genital herpes should be assessed for active lesions at the time of delivery.
Updated consensus guidelines for the management of women with abnormal cervical cytologic lesions and testing for HPV as well as the use of genotyping tests were published in 2013. The updated guidelines are discussed by Massad et al in The Journal of Lower Genital Tract Disease (including corrections from the original guidelines published), available on the website http://www.asccp.org/asccp-guidelines [199–201]. The guidelines are very comprehensive and present what is considered the optimal prevention strategies that would identify those HPV-related abnormalities likely to progress to invasive cancers while avoiding destructive treatment of

abnormalities not destined to become cancerous [202]. An updated consensus guideline frequently asked questions section is also available at http://www.asccp.org/consensus-guidelines-faqs. As with previous guidelines, HPV testing refers to validated HPV assays that have been analytically and clinically validated for cervical cancer and verified precancer cervical intraepithelial neoplasia 2+ by the FDA. Only testing for hrHPV types that are associated with cervical cancer is appropriate [202, 203]. Because the 2013 guidelines are lengthy, with 18 flowchart figures, essential changes and retained 2006 consensus guidelines are listed below.
Essential changes from prior management guidelines related to screening include:
Prior management guidelines from the 2006 consensus guidelines for the management of women with abnormal cervical screening tests [203] retained include:
Follow-up testing for abnormal cytology and/or positive hrHPV is complicated and readers are referred to the American Society for Colposcopy and Cervical Pathology guidelines for management decisions and the free teaching modules available (http://www.asccp.org/asccp-guidelines.
In 2015, additional interim clinical guidance papers were published for use of primary hrHPV testing for cervical cancer screening without Pap [201, 202]. An overview of possible advantages and disadvantages were addressed. Assessment from large databases showed major advantages in primary hrHPV screening as an alternative to current guidelines. Detection of hrHPV and genotypes 16/18 allowed triaging effectively as far as disease detection, number of screening tests, and overall colposcopies performed. Major disadvantages identified were a doubling of the number of colposcopies in ages 25–29 (thus why primary screening was recommended at age >30 years), and that primary hrHPV testing alone did not allow assessment of specimen adequacy that co-testing offered. A point-counterpoint on primary screening showed that this strategy is not yet fully accepted [203].
Patients with a cervix remaining after hysterectomy, HIV-infected patients, and patients that have received one of the HPV vaccines should undergo routine Pap and HPV screening and management. Testing should be postponed when a woman is menstruating [195, 202, 203].
In the United States, testing for syphilis is most commonly performed by serology and requires 2 tests. Traditionally testing has consisted of initial screening with an inexpensive nontreponemal test (ie, RPR), then retesting reactive specimens with a more specific, and more expensive, treponemal test (ie, Treponema pallidum particle agglutination). If a nontreponemal test is being used as the screening test, it should be confirmed, as a high percentage of false-positive results occur in many medical conditions unrelated to syphilis. When both test results are reactive, they indicate present or past infection. Many high-volume clinical laboratories have reversed the testing sequence and begin the testing algorithm first with a specific treponemal test, such as an EIA or chemiluminescence immunoassay, and then retesting reactive results with a nontreponemal test, such as RPR, to confirm diagnosis. Screening with a treponemal test can identify persons previously positive, treated, and/or partially treated for syphilis as well as yield false positives in patients with low likelihood of infection. If the follow-up confirmation test (RPR) is negative, it requires the laboratory to perform a different treponemal-specific test to guide management decisions (ie, fluorescent treponemal antibody–absorbed) [194, 204]. Treponema pallidum cannot be seen on Gram stain and cannot be cultured in the routine laboratory. Darkfield exam for motile spirochetes is unavailable in the majority of laboratories.
Chancroid, caused by the gram-negative organism H. ducreyi, lymphogranuloma venereum (LGV) caused by C. trachomatis serovars L1, L2, or L3, and granuloma inguinale (donovaniasis) caused by the intracellular gram-negative bacterium Klebsiella granulomatis, are genital ulcers uncommon in the United States and are typically diagnosed by clinical presentation, identification of high-risk factors, and exclusion of the more common genital lesions (syphilis and HSV). Chancroid may be identified by Gram stain and culture but is not recommended to be performed unless by a laboratory experienced in this testing. NAATs for C. trachomatis will detect LGV serovars but not specific serovars, but none are FDA-cleared for genital ulcer sites. Rectal swabs in patients with proctitis are recommended, and testing is available in laboratories that have validated this source [205].

B. Vaginosis/Vaginitis ::: XI. GENITAL INFECTIONS:
The diagnoses of bacterial vaginosis (BV), or altered vaginal flora, and vaginitis caused by fungal organisms (vulvovaginal candidiasis [VVC]) or Trichomonas vaginalis (TV), are often considered clinically and diagnostically as a group because of their overlapping signs and symptoms. However, the mode of transmission and/or acquisition is not necessarily that of an STI for VVC, but may be for BV and is for TV. A number of point-of-care tests can be performed from a vaginal

discharge specimen while the patient is in the healthcare setting. Although point-of-care tests are popular, the sensitivity and specificity for making a specific diagnosis vary widely and these assays, while rapid, are often diagnostically poor. Some of the tests include a pH strip test, scored Gram stain for BV, wet mount for TV, and 10% KOH microscopic examinations for VVC. For BV, use of clinical criteria (Amsel diagnostic criteria) is equal to a scored Gram stain of vaginal discharge. However, a scored Gram stain is more specific than probe hybridization, point-of-care tests, and culture that only detect the presence of G. vaginalis as the hallmark organism for altered vaginal flora) Table 38. For VVC and TV, the presence of pseudohyphae and motile trichomonads, respectively, allows a diagnosis. However, proficiency in microscopic examination is essential given that infections may be mixed and/or present with atypical manifestations. Unfortunately, consistent microscopic examination of vaginal specimens and interpretation are difficult for many laboratories to perform and wide variation of sensitivities (40%–70%) for both TV and VVC using smear examination exists relative to NAAT and culture, respectively [206]. It should be noted that recent publications utilizing NAATs highlight the prevalence of Trichomonas as equal to or greater than CT and GC in certain patient populations and point to a growing trend toward screening for TV, CT, and GC simultaneously [207, 208]. More recently, microbiome-based multiplex NAATs have become available for the diagnosis of BV and have been validated in several reference laboratories. One commercial product is now FDA-cleared. Preliminary data show greater specificity of this approach compared to methods that identify only G. vaginalis, as well as consistency in both reproducible as well as standardized results. Tests for the entities of vaginosis/vaginitis are shown in Table 38 [209–214].

GC Screening (Consider Local Epidemiology and Risk) ::: C. Urethritis/Cervicitis ::: XI. GENITAL INFECTIONS:
For laboratory diagnosis of CT and GC, NAATs are the preferred assays for detection because of increased sensitivity while retaining specificity in low-prevalence populations (pregnant patients) and the ability to screen with a noninvasive urine specimen [195]. Vaginal specimens in women (either provider or self-collected) and urine specimens in men are preferred specimen sources. In MSM, rectal and oropharyngeal testing is recommended. NAATs on samples other than genital (rectal, oropharyngeal, conjunctival) are currently not FDA-cleared and require in-house validation. Providers need to confirm with the laboratory if these sources will be tested. In general, retesting patients with a follow-up test for CT or GC (test of cure) is not recommended unless special circumstances exist (pregnancy, continuing symptoms). However, patients that are at higher risk for STIs should be screened within 3–12 months from the initial positive test for possible reinfection because those patients with repeat infections are at higher risk for PID. Requirements for testing practices and/or need for confirmatory testing in pediatric patients may vary from state to state, especially in potential victims of assault; check with state guidelines. Appropriate providers or laboratories that perform testing in children should be consulted [195].
Recently, prevalence studies using NAATs have shown that Trichomonas is as common as CT and more common that GC in certain clinical and geographic settings, with a uniquely high presence in women and men over 40 and in incarcerated populations. In addition, the ulcerative nature of TV infection leads to sequelae similar to those of CT and GC, including perinatal complications as well as susceptibility to HIV and HSV acquisition and transmission. FDA-cleared NAATs allow testing from the same screening specimens used for CT and GC testing, with significantly improved sensitivity over wet mount or hybridization test.

Mycoplasma genitalium is a recognized pathogen in nongonococcal urethritis and nonchlamydial nongonococcal urethritis in males and likewise cervicitis and PID in females. Fifteen percent to 25% of infections may be due to this organism, and resistance to first-line agents is rising [215, 216]. A NAAT may be the best option for detection of M. genitalium, due to issues with culture and cross-reactivity with serologic tests. While there is no FDA-cleared assay available, multiple laboratories have validated molecular assays. Culture or NAATs for Ureaplasma is not recommended because of the high prevalence of colonization in asymptomatic, sexually active people [195, 217].

D. Infections of the Female Pelvis ::: XI. GENITAL INFECTIONS:
Pelvic inflammatory disease (PID) is a spectrum of disorders and can be a serious infection in the upper genital tract/reproductive organs (uterus, fallopian tubes, and ovaries) and includes any single or combination of endometritis, tubo-ovarian abscess, and salpingitis. PID can be sexually

transmitted or naturally occurring, has the highest incidence in ages 15–25, and is the leading cause of infertility in women [218] (http://www.ashasexualhealth.org/stdsstis/pid/).
PID can be clinically difficult to identify when patients present with mild or nonspecific symptoms. Finding symptoms on physical examination (cervical motion tenderness) as well as other criteria (elevated temperature or mucopurulent discharge) increases the specificity and positive predictive value of laboratory tests. Diagnostic tests are dependent on the clinical severity of disease, epidemiological risk assessment, and whether invasive procedures, such as laparoscopy and/or endometrial biopsy, are used. Bacterial tests performed on non–aseptically collected specimens (endocervical or dilatation and curettage) have limited utility in diagnosing PID. Actinomyces spp are part of normal flora and can often be seen on Pap smears. While Actinomyces spp have been associated with intrauterine devices (IUDs) in the past, they are very uncommon and usually occur most commonly in 2 settings: if a patient has an infection at the time of insertion of an IUD and if the IUD is left in place past the recommended time of removal (typically 5 years) [219]. If Actinomyces infection is suspected, the laboratory should be notified to culture such samples anaerobically, including an anaerobic broth that is held for ≥5 days. Patients with suspected PID should be tested for CT, GC, and HIV. Both difficulty in diagnosis as well as significant potential sequelae should make the threshold for therapy low [220].
Postpartum endometritis should be suspected when the patient presents with high fever (≥101°F [38.3°C] or >100.4°F [38.0°C] on >2 occasions >6 hours apart after the first 24 hours of delivery and up to 10 days postdelivery), abdominal pain, uterine tenderness, and foul lochia. Usually a multiorganism syndrome, the infection is most commonly seen in patients with unplanned cesarean delivery because of the inability to introduce antibiotics quickly. Postpartum endometritis can be reduced by testing and treating for symptomatic BV late in pregnancy, which has been associated with preterm labor and prolonged delivery. Late postpartum endometritis suggests possible chlamydia or other chronic STI.
Although the role of culture in the setting of endometritis is controversial, diagnostic tests to consider in the diagnosis of PID and postpartum endometritis are shown in Table 40.

E. Special Populations ::: XI. GENITAL INFECTIONS:
Children for whom sexual assault is a consideration should be referred to a setting or clinic that specifically deals with this situation. Readers are referred to the references by Girardet et al and the 2015 CDC guidelines, where NAAT and noninvasive specimens have yielded excellent results [195, 221].
In MSM, the typical genital sites are not always infected (eg, the urethra or urine). Recommendations from the CDC now include screening in this population at a number of sites for GC and CT, including rectum, oropharynx, and urethra. Readers are referred to the CDC treatment guidelines for further recommendations [195] and a review of extragenital infections caused by CT and GC [221].
In pregnant patients, screening for HIV, syphilis, hepatitis B surface antigen, CT, and GC (if in high-risk group or high-GC-prevalence area) is routine. Symptomatic patients with vaginosis/vaginitis should be tested for BV and Trichomonas. Screening for group B streptococci (GBS) should occur at 35–37 weeks with both rectal and vaginal swab specimens submitted to optimize identification of carriers. Laboratories typically use an enrichment broth and selective media to enhance recovery for GBS. While NAATs are available for GBS, the sensitivity is optimal only when performed from an enrichment broth specimen. Women with bacteriuria with GBS (as single pathogen or predominant pathogen isolated) indicate high carriage and increased risk for transmission of GBS to the neonate. Treating GBS prior to 35–37 weeks does not eliminate the need to treat at the time of delivery, and patients are assumed to be carriers. Susceptibility testing of GBS is not routinely performed and recommended only if the patient is allergic to penicillin. Group A streptococci are not detected by GBS PCR tests. Past history of STIs, those in higher-risk groups, and/or clinical presentation consistent with infection, should be assessed for other pathogens as warranted (ie, HSV if vesicular lesions are present). Although rare, Listeria infection in the pregnant woman (usually acquired via ingestion of unpasteurized cheese or other food) can be passed to the fetus, leading to disease or death of the neonate. Due to nonspecific symptoms, diagnosis is difficult, but blood cultures from a bacteremic mother may allow detection of this pathogen in time for antibiotic prophylaxis Screening tests (serology, stool cultures) in pregnant women are not appropriate [222].

A. Burn Wound Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:

Reliance on clinical signs and symptoms alone in the diagnosis of burn wound infections is challenging and unreliable. Sampling of the burn wound by either surface swab or tissue biopsy for culture is recommended for monitoring the presence and extent of infection (Table 41). Quantitative culture of either specimen is recommended; optimal utilization of quantitative surface swabs requires twice-weekly sampling of the same site to accurately monitor the trend of bacterial colonization. A major limitation of surface swab quantitative culture is that microbial growth reflects the microbial flora on the surface of the wound rather than the advancing margin of the subcutaneous or deep, underlying damaged tissue. Quantitative bacterial culture of tissue biopsy should be supplemented with histopathological examination to better ascertain the extent of microbial invasion. Be advised that quantitative bacterial cultures may not be offered in all laboratories; quantitative biopsy cultures should be considered for patients in whom grafting is necessary. For laboratories that provide quantitative wound culture services to wound care centers, which predominately manage chronic wounds, obtaining clinically relevant results is dependent upon obtaining tissue from deep within the wound to avoid surface and subsurface microbial flora, which essentially colonize these areas and are part of a biofilm. Collection of specimens using swabs is discouraged due to the significant limitations of swabs: (1) high risk of contamination with surface and subsurface contamination, and (2) limited specimen capacity (500 µL) leading to insufficient quantity of specimen, especially when cultures (fungal, mycobacterial) other than bacteriology are requested. Prior to any sampling or biopsy, the wound should be thoroughly cleansed and devoid of topical antimicrobials and debris that can affect culture results. Blood cultures should be collected for detection of systemic disease secondary to the wound. The application of NAAT for detection of listed viruses is commonly restricted to blood and/or body fluids. It is advisable that the clinician determine if the local supporting laboratory has validated such assays and if the laboratory has assessed the performance with tissue specimens. This precaution would also apply to the molecular detection of MRSA (except for one FDA-cleared test for S. aureus and MRSA from SSTIs) and VRE [224, 225].

B. Human Bite Wound Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
The human oral cavity contains many potential aerobic and anaerobic pathogens and is the primary source of pathogens that cause infections following human bites. The most common of these are Staphylococcus spp, Streptococcus spp, Clostridium spp, pigmented anaerobic gram-negative rods, and Fusobacterium spp. Such infections are common in the pediatric age group and are often inflicted during play or by abusive adults. Bite wounds can vary from superficial abrasions to more severe manifestations including lymphangitis, local abscesses, septic arthritis, tenosynovitis, and osteomyelitis. Rare complications include endocarditis, meningitis, brain abscess, and sepsis with accompanying disseminated intravascular coagulation, especially in immunocompromised patients.
In addition to the challenge of acquiring a representative wound specimen for aerobic and anaerobic culture, a major limitation of culture is the potential for misleading information as a result of the polymicrobial nature of the wound. It is important that a Gram stain be performed on the specimen to assess the presence of indicators of inflammation (eg, neutrophils), superficial contamination (squamous epithelial cells), and microorganisms. Swabs are not the specimen of choice in many cases (Table 42). Major limitations of swabs vs tissue biopsy or aspirates include (1) greater risk of contamination with surface/colonizing flora; (2) limited quantity of specimen that can be acquired; (3) drying unless placed in appropriate transport media, which in itself dilutes out rare microbes and further limits the yield of the culture [226–228].

C. Animal Bite Wound Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
As with human bite wounds, the oral cavity of animals is the primary source of potential pathogens and thus the anticipated etiological agent(s) is highly dependent upon the type of animal that inflicted the bite (Table 43). As dogs and cats account for the majority of animal-inflicted bite wounds, the 2 most prominent groups of microorganisms initially considered in the evaluation of patients are Pasteurella spp, namely P. canis (dogs) and P. multocida subsp multocida and subsp septica (cats) or Capnocytophaga canimorsus. Other common aerobes include streptococci, staphylococci, Moraxella spp, and saprophytic Neisseria spp. Animal bite wounds are often polymicrobial in nature and include a variety of anaerobes. Due to the complexity of the microbial flora in animals, examination of cultures for organisms other than those listed in Table 43 is of little benefit since these organisms are not included in most of the commercial identification systems (conventional and automated) databases [229–238]. Matrix-assisted laser desorption–ionization mass spectrometry has proven valuable in identifying

organisms when conventional phenotypic systems have failed. If rabies or herpes B infection is suspected, contact the local or state public health laboratory for assistance and advice on how to proceed.

D. Trauma-Associated Cutaneous Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
Infections from trauma are usually caused by exogenous or environmental microbial flora but can be due to the individual's endogenous (normal) flora (Table 44). It is strongly recommended that specimens not be submitted for culture within the first 48 hours posttrauma as growth from specimens collected within this time frame most likely represents environmental flora acquired at the time of the trauma episode (motor vehicle accident, stabbings, gunshot wounds, etc). The optimal time to acquire cultures is immediately after debridement of the trauma site [239–242]. It is strongly recommended that initial cultures focus on common pathogens, with additional testing being reserved for uncommon or rare infections associated with special circumstances (eg, detection of Vibrio spp following saltwater exposure) or patients with chronic manifestations of infection or who do not respond to an initial course of therapy.
Although not considered in quite the same manner as external trauma, intravenous drug users inject themselves with exogenous substances that may include spores from soil and other contaminants that cause skin and soft tissue infections, ranging from abscesses to necrotizing fasciitis. Agents are similar to those in Table 44, with the addition of Clostridium sordellii, C. botulinum (causing wound botulism), and the agents of human bite wounds (Table 42) among skin poppers who use saliva as a drug diluent.

E. Surgical Site Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
Surgical site infections (SSIs) may be caused by endogenous flora or originate from exogenous sources such as healthcare providers, the environment, or materials manipulated during an "incisional" or "organ/space" surgical procedure. Incisional infections are further divided into superficial (skin and subcutaneous tissue) and deep (tissue, muscle, fascia). Deep incisional and organ/space infections are the SSIs associated with the highest morbidity. The reader is referred to the CDC guidelines for prevention of surgical site infections, 2014, for specific definitions of SSIs (http://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf). Of the microbial agents listed below (Table 45), S. aureus, including MRSA, coagulase-negative staphylococci, and enterococci are isolated from nearly 50% of these infections [243]. Although enterococcal species are commonly isolated from superficial cultures, they are seldom true pathogens; regimens that do not include coverage for enterococci are successful for surgical site infections. To optimize clinically relevant laboratory results, resist the use of swabs during surgical procedures, and instead submit tissue, fluids, or aspirates.

F. Interventional Radiology and Drain Devices ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
Common interventional devices that are used for diagnostic or therapeutic purposes include interventional radiology and surgical drains. The former consists of minimally invasive procedures (angiography, balloon angioplasty/stent, chemoembolization, drain insertions, embolizations, thrombolysis, biopsy, radiofrequency ablation, cryoablation, line insertion, inferior vena cava filters, vertebroplasty, nephrostomy placement, radiologically inserted gastrostomy, dialysis access and related intervention, transjugular intrahepatic portosystemic shunt, biliary intervention, and endovenous laser ablation of varicose veins) performed using image guidance. Procedures are regarded as either diagnostic (eg, angiogram) or performed for treatment purposes (eg, angioplasty). Images are used to direct procedures that are performed with needles or other tiny instruments (eg, catheters). Infections as a result of such procedures are rare but should be considered when evaluating a patient who has undergone interventional radiology, which constitutes a risk factor for infection due to the invasive nature of the procedure.
A variety of drainage devices are used to remove blood, serum, lymph, urine, pus, and other fluids that accumulate in the wound bed following a procedure (eg, fluids from deep wounds, intracorporeal cavities, or intra-abdominal postoperative abscess). They are commonly used following abdominal, cardiothoracic, neurosurgery, orthopedic, and breast surgery. Chest and abdominal drains are also used in trauma patients. The removal of fluid accumulations helps to prevent seromas and their subsequent infection. The routine use of postoperative surgical drains is diminishing, although their use in certain situations is quite necessary.
The type of drain to be used is selected according to quality and quantity of drainage fluid, the amount of suction required, the anatomical location, and the anticipated amount of time the drain will be needed. Tubing may also be tailored according to the aforementioned specifications. Some

types of tubing include round or flat silicone, rubber, Blake/channel, and triple-lumen sump. The mechanism for drainage may depend on gravity or bulb suction, or it may require hospital wall suction or a portable suction device. Drains may be left in place from 1 day to weeks, but should be removed if an infection is suspected. The infectious organisms that may colonize a drain or its tubing typically depend on the anatomical location and position of the drain (superficial, intraperitoneal, or within an organ, duct or fistula) and the indication for its use. Interpretation of culture results from drains that have been in place for >3 days may be difficult due to the presence of colonizing bacteria and yeast.
Drains are characterized as gravity, low-pressure bulb evacuators, spring reservoir, low pressure, or high pressure. Fluids from drains are optimal specimens for collection and submission to the microbiology laboratory. All fluids should be collected aseptically and transported to the laboratory in an appropriate device such as blood culture bottle (aerobic), sterile, leak-proof container (ie, urine cup), or a citrate-containing blood collection tube to prevent clotting in the event that blood is present. Expected pathogens from gravity drains originate from the skin or gastrointestinal tract; for the remaining drain types, skin flora represent the predominate pathogens.

G. Cutaneous Fungal Infections ::: XII. SKIN AND SOFT TISSUE INFECTIONS:
The presence of fungi (molds or yeasts) on the skin poses a challenge to the clinician in determining if this represents contamination, saprophytic colonization, or is a true clinical infection. For convenience, the fungi have been listed by the type of mycosis they produce (Table 46): for example, dermatophytes typically produce tinea (ringworm)–type infections; dematiaceous (darkly pigmented molds and yeast-like fungi) cause both cutaneous and subcutaneous forms of mycosis; dimorphic fungi generally cause systemic mycosis and the presence of cutaneous lesions signifies either disseminated or primary (direct inoculation) infection; yeast-like fungi are usually agents of opportunistic types of mycoses but can also manifest as primary or disseminated disease as is true for the opportunistic molds (eg, Aspergillus spp, Fusarium spp). In addition to the recommended optimal specimens and associated cultures, fungal serology testing (complement fixation and immunodiffusion performed in parallel, not independent of the other) is often beneficial in diagnosing agents of systemic mycosis, specifically those caused by Histoplasma and Coccidioides. In cases of active histoplasmosis and blastomycosis, the urine antigen test may be of value in identifying disseminated disease.
The clinician should be aware that dematiaceous fungi (named so because they appear darkly pigmented on laboratory media) do not always appear pigmented in tissue but rather hyaline in nature. To help differentiate the dematiaceous species, a Fontana-Masson stain (histopathology) should be performed to detect small quantities of melanin produced by these fungi. It is not uncommon for this group of fungi to be mistakenly misidentified by histology as a hyaline mold such as Aspergillus spp. This highlights the importance of correlating culture results with histological observations in determining the clinical relevance since the observation of fungal elements in histopathology specimens is most likely indicative of active fungal invasion [244, 245].

XIII. ARTHROPOD-BORNE INFECTIONS:
The clinical microbiology tests of value in establishing an etiology of various arthropod-borne diseases are presented below. Those transmitted by ticks are most likely to require clinical laboratory support (Table 47). Borreliosis includes relapsing fever, Borrelia miyamotoi infection, and Lyme borreliosis; these diseases are transmitted by ticks to humans. Lyme borreliosis or Lyme disease (primarily due to infection with Borrelia burgdorferi or Borrelia mayonii in the United States), a multisystem disease that can affect the skin, nervous system, joints, and heart, is the most frequently reported tick-borne disease in the northern hemisphere [246]. Most commonly, early localized Lyme disease (LD) is diagnosed on clinical grounds, including the presence of erythema migrans. Erythema migrans (EM; an expanding rash) was previously considered pathognomonic for Lyme borreliosis; however, other infections can mimic this dermatologic presentation (eg, southern tick-associated rash illness, cellulitis). Diagnostic testing for LD in patients who present with a characteristic EM rash, alongside an appropriate exposure history, is contraindicated, as antibodies to B. burgdorferi may not yet be detectable, leading to false-negative results and undertreatment. While NAAT for LD-associated Borrelia spp is available through multiple reference laboratories, performance of this testing on whole blood or other blood fractions for detection of early disseminated or late stages of LD is not recommended due to low sensitivity in this specimen source.

A notable exception to this is NAAT for B. mayonii; this newly described agent of LD is associated with a higher level of spirochetemia, and given the lack of serologic assays able to detect specific antibodies to this species, NAAT of whole blood is recommended for detection of B. mayonii [247]. For atypical EM, serology may be obtained and, if negative, one may obtain a convalescent serum or continue observation to see if the EM becomes more characteristic. Otherwise, NAATs may be performed on EM biopsy specimens to confirm early localized LD if the visual appearance of the EM rash is questionable. Serologic testing using a 2-tiered testing algorithm (TTTA) remains the testing methodology of choice for both early disseminated and late stages of LD. The TTTA currently recommended by the CDC involves an initial EIA or indirect fluorescent antibody (IFA) screen for antibodies to LD-associated Borrelia spp, followed by supplemental Western blot or immunoblot testing for specific IgM- and IgG-class antibodies to B. burgdorferi in any sample positive or equivocal by an EIA/IFA screen. Immunoblot testing for antibodies to B. burgdorferi should not be performed as a standalone test as specificity is reduced.

Borrelia burgdorferi–specific IgG and IgM scoring is based on the presence of at least 5 out of a possible 10 diagnostic IgG bands and 2 of a possible 3 IgM bands following reaching the threshold of a positive or equivocal screening EIA [248]. The IgM blot is not clinically meaningful in patients who present 30 days or longer after symptom onset due to high rates of false positivity. Additionally, seropositivity for both IgM- and IgG-class antibodies to LD-associated Borrelia spp may persist for months to years (>10–15 years) following resolution of the infection [249]. Since positivity by the TTTA may reflect remote exposure rather than current infection, it is recommended that only symptomatic patients with an appropriate exposure history be tested for LD. Finally, multiple LD “specialty” laboratories have emerged in recent years, claiming expertise in tick-borne disease diagnosis and offering LD diagnostic assays with improved sensitivity [250, 251]. These laboratories may not be CLIA-approved and offer LD diagnostic assays using methods and interpretive criteria for which validation data has neither been made publically available nor been vetted by high-quality peer review. Submission of patient specimens to such laboratories is not recommended.

Classical relapsing fever transmitted by the bites of soft (argasid) ticks burdens residents or travelers to mainly the western United States, although sporadic cases occur in the south-central states. Louse-borne relapsing fever is endemic to tropical countries or may become epidemic in refugee camps; travelers would be the only patients who might present with louse-borne relapsing fever, and their diagnosis would be similar to that for tick-borne relapsing fever. Relapsing fever presents as recurrent fevers of several days’ duration, terminating with crisis and resuming after a few days; febrile episodes are marked by the presence of large numbers of spirochetes in the peripheral blood. Relapsing fever-like borreliae (B. miyamotoi) transmitted by the same hard tick as that transmitting the agents of LD cause fever that has a less characteristic presentation and may be confused with human granulocytic anaplasmosis; spirochetes are sparse in peripheral blood but are usually detectable by NAAT (particularly reverse-transcription PCR [RT-PCR]). Recent data suggest that both acute and convalescent sera from patients with B. miyamotoi infection are frequently reactive by first-tier serologic assays for LD (eg, C6 EIA) and convalescent sera may be positive of B. burgdorferi–specific IgM blots [252]. Despite this, testing for B. miyamotoi infection using B. burgdorferi serologic assays is not recommended.

In the United States, rickettsial diseases that are transmitted by ticks include Rocky Mountain spotted fever (RMSF) due to Rickettsia rickettsii; “mild” RMSF (Rickettsia parkeri and other spotted fever group Rickettsia spp), human granulocytic anaplasmosis (Anaplasma phagocytophilum), human monocytic ehrlichiosis (Ehrlichia chaffeensis), and ehrlichiosis caused by Ehrlichia ewingii or Ehrlichia muris [253, 254]. Although clinically similar, these diseases are epidemiologically and etiologically distinct illnesses. Endemic typhus and flea-borne typhus (Rickettsia typhi and Rickettsia felis, respectively) may also infect people in the United States, mainly in warmer sites where fleas are common throughout the year. Rare epidemic typhus (Rickettsia prowazekii) cases have been recorded in the United States from contact with flying squirrels or their nests. Rickettsialpox (Rickettsia akari), comprising a mild febrile disease with rash and eschar, is maintained by mouse mites in many large urban areas. The diagnosis of patients with these infections is challenging early in the course of their clinical infection since signs and symptoms are often nonspecific or mimic benign viral illnesses. Rash is usually present in most acute rickettsiosis, but skin color may prevent its recognition. The likelihood of severe morbidity or mortality with delaying treatment for RMSF means that patients should be presumptively treated without waiting for laboratory confirmation, which rests mainly on seroconversion.

In addition to borreliosis and rickettsial diseases, babesiosis, tularemia, Powassan/deer tick virus encephalitis, and Colorado tick fever virus are also transmitted by ticks in the United States. While not yet officially confirmed, emerging viruses, such as Heartland virus and Bourbon virus, are also strongly suspected to be transmitted to humans via tick vectors. With the exception of babesiosis, which may comprise as much as a third as many cases as Lyme borreliosis in some sites, these other tick-borne infections are relatively rare (a tenth as common as Lyme borreliosis). Annually, tick-transmitted viral infections are on the order of 25 or fewer cases a year nationally; however, this is likely an underrepresentation due to the lack of available clinical assays for routine detection of these emerging vector-borne infections. Most cases due to these less common infections present with fever >38.9°C (>102°F) but other than the arboviral infections with neurologic signs, the presentation is nonspecific. Other than the use of NAAT and assessment of blood smears for detection of Babesia spp, laboratory confirmation of a diagnosis of these less common infections depends on seroconversion.
As the most of the organisms transmitted by ticks are infrequently encountered in clinical specimens, many clinical microbiology laboratories do not provide all of the services listed in the table below. Of significance, while relapsing fever, ehrlichiosis, anaplasmosis, and babesiosis can all be rapidly diagnosed by examining peripheral blood smears, a negative smear result does not necessarily rule out these tick-borne infections due to the often low and variable sensitivity of a peripheral blood smear examination. Leukopenia, thrombocytopenia, and elevated liver enzymes may also help establish the need for specifically testing for these tick-borne infections.
Body lice may transmit the agent of trench fever (Bartonella quintana), and fleas that of diverse bartonelloses, including cat scratch disease due to Bartonella henselae. Transmission may occur by bites of these arthropods, but a more likely mode of exposure is to the infectious louse or flea excreta. The bartonelloses may present as acute febrile disease, with or without lymphadenopathy. These gram-negative bacteria are fastidious and slow growing, requiring hemin and a humidified carbon dioxide atmosphere. If lymphadenopathy is present, aspirates may be cultured; whole blood needs to be lysed for effective cultivation. NAAT is more sensitive and rapid. The IFA test may confirm infection, particularly if seroconversion is documented; there is significant IgG cross-reactivity between the bartonellae, though, and thus specific identification of the infecting species may not be possible without cultures or NAATs.
While laboratory identification of arthropods submitted by patients can provide limited information with respect to exposure risk, testing of these arthropods for the presence of infectious agents has no clinical value. For example, testing of engorged or partially engorged ticks for tick-borne infectious organisms should be avoided as the presence of an organism (via nucleic acid detection) in a tick does not indicate that the infectious agent was transmitted to the patient. Symptomatic patients, not the removed arthropod, should be tested for specific vector-borne infections, guided by clinical presentation, duration of symptoms, and exposure history.
For all of the arthropod-borne infections, clinical specimens for culture, molecular analysis, and the majority of serologic assays are, for the most part, sent to reference laboratories. In addition, because most NAATs for the diseases listed are not FDA-cleared, such tests are not universally available. As with most infections, paired serologic testing of patients suspected of having a tick-borne disease, with samples collected at presentation and at 3–4 weeks of follow-up, provide the best probability of confirming a diagnosis. With these limitations in the availability of and performance of various testing formats (ie, culture, molecular analysis, and the majority of serologic assays), the provider needs to check with the laboratory for availability of testing, the optimum testing approach, appropriate specimen source, and turnaround time.
Key points for the laboratory diagnosis of arthropod-borne infections:

A. Human Immunodeficiency Virus ::: XIV. VIRAL SYNDROMES:
HIV type 1 (HIV-1) is an RNA virus with a genome consisting of 3 major genes encoding capsid proteins (gag p55, p24, and p17), reverse transcriptase, protease, integrase (pol p66, p51, and p31), and envelope glycoproteins (env pg160, gp120, and gp41). HIV viruses are classified based on the relatedness of their genome into types 1 and 2, groups, and clades. HIV-1 is categorized into groups M, O, and P, with M being most common [264, 265]. HIV-1 is more common than HIV type 2 (HIV-2) in the United States, but the latter should be considered in persons who were born in, have traveled to, have received blood products from, or have had a sexual partner from West Africa, as well as those who have been similarly exposed to HIV-2–infected persons in any geographic area.
After exposure to HIV, HIV RNA is detectable in plasma by 10–12 days, followed by appearance of HIV p24 antigen in serum or plasma at 15–17 days. Depending on the sensitivity of the serologic

assays used, HIV-specific antibodies are detectable in serum or plasma at the earliest at 21 days after exposure. Performing an HIV RNA test after a negative initial antibody and/or antigen test in persons suspected of acute infection may therefore be helpful. Due to the time course of test positivity and the possibility of seronegativity, laboratory diagnosis of primary (acute) HIV-1 infection is usually based on a high quantitative HIV-1 RNA (viral load) result (typically >105 copies/mL) or qualitative detection of HIV-1 RNA and/or proviral DNA (Table 48) [266]. However, in the setting of nonacute HIV infection, HIV viral load assays should be used with caution for diagnosis of HIV infection because of the possibility of false-positive results. Since false-positive results are generally of low copy number (<1000 copies/mL), low copy number results should prompt retesting of a second specimen. Notably, because there is a 10- to 12-day period after infection when serologic markers are not detectable, testing another specimen 2–4 weeks later should be considered if initial antibody, antigen, or RNA tests are negative. NAAT is not 100% sensitive in individuals with established HIV infection due to viral suppression, either naturally or therapeutically, or improper specimen collection/handling. If NAAT is used to make a diagnosis of acute HIV-1 infection, subsequent HIV-1 seroconversion by conventional serologic testing is recommended.

In the neonate, serologic testing is unreliable due to persistence of maternal antibodies; quantitative HIV-1 RNA testing is as sensitive as qualitative HIV-1 RNA and/or proviral DNA testing for the diagnosis of HIV-1 infection [267]. NAAT is recommended at 14–21 days, 1–2 months, and 4–6 months after birth, in infants born to HIV-1–infected mothers. Since the availability of HIV serologic assays in the 1980s, HIV screening tests have evolved to the current fourth- and fifth-generation assays in which recombinant and synthetic HIV peptide antigens are used in the detection of HIV p24 antigen and specific IgM and IgG antibodies. Such assays generally yield positive results by 4–6 days after positive NAAT results. Fifth-generation screening assays have the advantage over fourth-generation assays in their ability to discriminate among HIV-1 p24 antigen, HIV-1 antibodies, and HIV-2 antibodies.

HIV-1 p24 antigen is detected in serum or plasma usually by 14–16 days after infection (before antibody becomes detectable), and it typically decreases below detection limits thereafter, limiting the utility of p24 antigen testing alone for the diagnosis of HIV infection. The US Association of Public Health Laboratories and the CDC now recommend the use of fourth-generation assays for initial screening of individuals for diagnosis of HIV infection [264, 265]. The testing algorithm using such assays recommends that serum or plasma specimens with reactive screening test results be tested in reflex with HIV antibody differentiation immunoassays that can distinguish between HIV-1 and HIV-2 antibodies. If the antibody differentiation assay result is negative, further testing with a qualitative or quantitative NAAT is recommended to rule out acute HIV-1 infection. If the differentiation assay is positive, viral load testing (and usually also CD4 cell count determination) is recommended to direct management. Alternatively, if a fifth-generation HIV antigen/antibody combination assay is used as the initial test and if only the HIV p24 antigen is reactive, then such specimens can be tested subsequently by NAAT, whereas those specimens that are reactive for HIV-1 or HIV-2 antibodies can be tested subsequently with HIV antibody differentiation assays. Use of third-generation screening assays is limited to the diagnosis of nonacute HIV-1 infection (Table 48), since these assays can detect only HIV-1 and HIV-2 antibodies. Serum or plasma specimens that are reactive with such screening assays can be tested further with HIV antibody differentiation assays for confirmation. Individuals with initially reactive results in whole blood, serum, plasma, or saliva tested with rapid HIV antibody-only or antigen-antibody assays (eg, point-of-care rapid tests) should be tested further by laboratory-based fourth- or fifth-generation HIV immunoassays to determine the HIV infection status as described above. The current Association of Public Health Laboratories/CDC HIV testing algorithm no longer recommends supplemental HIV-1 antibody Western blot testing because of the subjectivity, labor intensity, and limited access of this manual assay.

Antiviral drug resistance testing is recommended for patients with acute or chronic HIV infection prior to initiating therapy (including treatment-naive pregnant HIV-1–infected women), virologic failure during combination drug therapy, and suboptimal suppression of viral load after initiating therapy. Genotypic resistance testing is recommended generally for treatment-naive patients, while phenotypic resistance testing is reserved mainly for treatment-experienced patients whose genotypic HIV resistance profiles show multiple resistance-associated mutations that could not predict an effective antiviral drug combination.

B. Epstein-Barr Virus ::: XIV. VIRAL SYNDROMES:

Epstein-Barr virus is a cause of mononucleosis among immunocompetent individuals and lymphoproliferative disease in immunocompromised patients. An elevated white blood cell count with an increased percentage of atypical lymphocytes is common in EBV-associated mononucleosis. Heterophile antibodies usually become detectable between the sixth and tenth day following symptom onset, increase through the second or third week of the illness and, thereafter, gradually decline over a year or longer. False-positive heterophile antibody results may be observed in patients with autoimmune disorders, leukemia, pancreatic carcinoma, viral hepatitis, or CMV infection. False-negative results are obtained in approximately 10% of patients, and are especially common in children younger than 4 years.
When the results of rapid Monospot or heterophile testing are negative, additional laboratory testing (Table 49) may be considered to differentiate EBV infection from a mononucleosis-like illness caused by CMV, HIV, or Toxoplasma gondii. In this situation, EBV-specific antibody testing for IgG- and IgM-class antibodies to the viral capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA) is recommended. The presence of VCA IgM (with or without VCA IgG) antibodies in the absence of IgG antibodies to EBNA suggests recent, primary infection with EBV. The presence of anti-EBNA IgG antibodies indicates that infection occurred at least 6–12 weeks prior, and therefore, is suggestive of a past (remote) infection with EBV. IgG-class antibodies to EBNA generally develop 2–3 months after primary infection and are detectable for life. Over 90% of the adult population has IgG-class antibodies to VCA and EBNA antigens, although approximately 5%–10% of patients who have been infected with EBV fail to develop antibodies to EBNA.
EBV is associated with lymphoproliferative disease in patients with congenital or acquired immunodeficiency, including patients with severe combined immunodeficiency, recipients of organ or peripheral blood stem cell transplants, and patients infected with HIV. An increase in the EBV viral load in peripheral blood or plasma, as measured by a quantitative NAAT, may occur in patients before the development of EBV-associated lymphoproliferative disease. Viral loads should be measured no more frequently than once per week, and these levels typically decrease with effective therapy. A difference in the viral load of ≥0.5 log10 between samples, preferably evaluated by the same assay, is typically required to demonstrate a significant change.
Conversion of EBV copies/mL to IU/mL using the World Health Organization (WHO) standard (or a WHO traceable standard) allows for laboratory-to-laboratory comparison of results. Tissues from patients with EBV-associated lymphoproliferative disease may show monoclonal, oligoclonal, or polyclonal lesions. The diagnosis of EBV-associated lymphoproliferative disease (eg, posttransplant lymphoproliferative disorder) requires multiple tests, including quantitative NAAT, radiology (eg, positron emission tomography scan), and detection of EBV DNA, RNA, or protein in biopsy tissue.
NAATs may be used to detect EBV DNA in CSF of patients with AIDS-related CNS lymphoma. However, EBV DNA may also be present in the CSF of patients with other abnormalities (eg, CNS toxoplasmosis, pyogenic brain abscesses), and therefore, positivity is nondiagnostic. Detection of EBV-specific antibodies in CSF may indicate CNS infection; however, it may also be observed if the CSF fluid becomes contaminated with blood during collection, or if there is transfer of antibodies across the blood–brain barrier. Calculation of the CSF-to-serum antibody index may be helpful, but this type of testing is not performed in most clinical laboratories.

C. Cytomegalovirus ::: XIV. VIRAL SYNDROMES:
Cytomegalovirus is a member of the Herpesviridae family and causes acute and latent infection. Infection with CMV is very common, resulting in mild or asymptomatic disease in most immunocompetent individuals. However, CMV is a significant cause of morbidity and mortality among immunocompromised hosts, especially transplant recipients. Serologic testing for CMV-specific antibodies is typically limited to pretransplant screening of the donor and recipient (Table 50). This is usually accomplished by testing for anti-CMV IgG-class antibodies, which, when present, indicate past exposure to CMV. The utility of testing for IgM-class antibodies is more limited, and may serve as an adjunct in the diagnosis of recent CMV infection; however, false-positive CMV IgM results may occur in patients infected with EBV or with immune disorders.
In recipients of solid organ or peripheral blood stem cell transplants, monitoring CMV viral loads by a quantitative NAAT is used to diagnose CMV-associated signs and symptoms, to guide preemptive treatment, and to monitor response to antiviral therapy. For laboratories using LDTs, Standard Reference Material (SRM) is available from the National Institute of Standards and Technology for CMV viral load measurement. SRM 2366, which consists of a bacterial artificial chromosome that contains the genome of the Towne strain of CMV, is used for assignment of the number of amplifiable genome copies of CMV/volume (eg, copies/µL). However, 4 FDA-approved

assays (Abbott RealTime CMV, Abbott Molecular, Inc; artus CMV RGQ MDx Kit, Qiagen, Inc; Cobas AmpliPrep/Cobas TaqMan CMV Test, Roche Molecular Systems, Inc; Cobas CMV, Roche Molecular Systems, Inc) are now available that are calibrated against the WHO standard and allow for normalization of results to international units per milliliter. Conversion of copies/mL to IU/mL using the WHO standard (or a WHO traceable standard) allows for laboratory-to-laboratory comparison of results.

Cytomegalovirus can be cultured from peripheral blood mononuclear cells (and other clinical specimens). However, isolation is labor-intensive and can take up to 14 days. The turnaround time can be reduced to 1–2 days with the use of the shell vial assay. In addition to a long turnaround time, culture-based assays have poor sensitivity for the recovery of CMV. Because the viral load is typically high and CMV is shed in the urine of newborns, urine culture for CMV continues to be used at some institutions for the diagnosis of congenital CMV infection.

Cytomegalovirus antigens can be demonstrated by immunohistochemical or in situ hybridization tests of formalin-fixed, paraffin-embedded tissues. Cytomegalovirus DNA, detected using NAAT in a variety of clinical specimens, may be useful in diagnosing CMV disease.

Among immunocompromised patients with CMV infection, the potential exists for the emergence of resistance to antiviral agents. A variety of assays can be used to assess antiviral resistance, most commonly by sequencing of the UL97 (phosphotransferase gene) and UL54 (DNA polymerase gene) genes. Sequencing-based assays are performed on DNA amplified directly from clinical specimens, provided they contain a sufficient quantity of CMV DNA. Alternatively, the virus can first be isolated in cell culture. Ganciclovir resistance most commonly emerges due to point mutations or deletions in UL97 (with foscarnet and cidofovir unaffected), with mutations at 3 codons (460, 594, 595) being most common. UL54 point mutations or deletions occur less frequently. If UL54 mutations are selected by ganciclovir or cidofovir, there is typically cross-resistance to both ganciclovir and cidofovir but not foscarnet. However, if mutations are selected by foscarnet, there is usually no cross-resistance to ganciclovir or cidofovir.

NAATs may be used to detect CMV DNA in CSF of patients with suspected CMV CNS infection, but false-positive results may occur (eg, in patients with bacterial meningitis in whom CMV DNA in blood crosses the blood–brain barrier and contaminates CSF). Detection of antibodies in CSF may indicate CNS infection; however, it may also be observed if the CSF fluid becomes contaminated with blood during collection, or if there is transfer of antibodies across the blood–brain barrier.

D. Varicella Zoster Virus ::: XIV. VIRAL SYNDROMES:

Varicella zoster virus is a member of the Herpesviridae family and causes chickenpox and shingles (zoster). Serology is not usually recommended for the diagnosis of acute disease, but the presence of anti-VZV IgM antibodies typically indicates recent exposure to VZV. However, an elevated IgM response may also be observed in patients with recent immunization to VZV or reactivation of latent virus. A positive VZV IgG with a negative IgM result suggests previous exposure to VZV and/or response to vaccination. A negative IgG result coupled with a negative IgM result indicates the absence of prior exposure to VZV and no immunity, but does not rule out VZV infection as the serum specimen may have been collected before the appearance of detectable antibodies. Negative results in suspected early VZV infection should be followed by testing a new serum specimen in 2–3 weeks.

Although viral culture can be used to recover VZV from clinical specimens, it may take up to 14 days for cytopathic effect to be observed. Due to this delay in turnaround time, NAATs have become routinely used for the diagnosis of VZV and offer the most sensitive and rapid approach to detect the virus (Table 51). For dermal lesions that are suspected to be associated with VZV infection, a culture transport swab should be vigorously rubbed on the base of the suspect skin lesion; the vesicle may be unroofed to expose the base. The swab should then be placed in viral transport media and transported to the testing laboratory. A less sensitive method for diagnosis is detection of viral antigens by direct fluorescent antibody stain of lesion scrapings. Suspected VZV-associated skin lesions should be clinically differentiated from smallpox. Information regarding clinical manifestations of smallpox, including differentiation from VZV pocks, and laboratory testing can be found on the CDC website (https://www.cdc.gov/smallpox/index.html).

VZV NAATs can be performed on CSF as an aid to the diagnosis of VZV CNS infection. Detection of anti-VZV IgM antibodies in the CSF may also be used to support a diagnosis of VZV meningoencephalitis, but if performed, should be completed alongside evaluation of anti-VZV levels in serum and NAAT in CSF.

E. Herpes Simplex Virus ::: XIV. VIRAL SYNDROMES:
Herpes simplex virus types 1 (HSV-1) and -2 (HSV-2) are common causes of dermal and genital lesions, but may also result in CNS disease or congenital infections. Serology should not be used a primary diagnostic test, but may assist in determining a patient's exposure status to HSV-1/2. The presence of IgG-class antibodies to the HSV-1/2 glycoprotein G antigen indicates previous exposure to the corresponding serotype of the virus. Positive IgG results do not differentiate past from current, active infection unless seroconversion is determined by testing acute and convalescent phase specimens. A 4-fold increase in anti-HSV IgG levels may suggest recent exposure; however, most commercial assays no longer yield a titered result that can be used quantitatively. The presence of IgM-class antibodies to HSV suggests primary infection; however, anti-HSV IgM reactivity is often absent at the time of lesion development, with IgM seroconversion occurring 1–2 weeks after infection. Also, commercial IgM assays are not able to reliably distinguish between infection with HSV-1 and HSV-2, and may be falsely positive due to other viral infections, alloantibodies present during pregnancy, or autoimmune disorders.
NAAT is the most sensitive, specific, and rapid test for diagnosis of HSV-associated skin or mucosal lesions and can detect and distinguish HSV-1/2 (Table 52). For collection of specimens, a viral culture transport swab should be vigorously rubbed over the base of the suspect skin or mucosal lesion; the vesicle may be unroofed to expose the base. Older, dried, and scabbed lesions are less likely to yield positive results. Culture and direct fluorescent antibody testing are less sensitive than NAATs, especially for the detection of HSV-1/2 from CSF.
HSV NAATs are now considered the gold standard to diagnose HSV CNS disease [268]. The assay should detect and distinguish HSV-1/2; type 1 is most commonly associated with encephalitis and type 2 with meningitis. Viral culture of CSF is insensitive for diagnosis of HSV CNS disease and should not be used to rule out HSV encephalitis/meningitis.

F. Human Herpesvirus Type 6 ::: XIV. VIRAL SYNDROMES:
Human herpes virus type 6 causes roseola infantum in children and can cause primary infection or reactivation in immunocompromised patients. Although serologic testing is not the preferred means of establishing a diagnosis of HHV-6 infection, IgG seroconversion, the demonstration of anti–HHV-6 IgM, or a 4-fold rise in IgG antibody titers using paired sera may indicate recent infection. Commercial assays do not typically distinguish between variants A and B. Because of the ubiquitous nature of HHV-6, most people have been exposed to the virus by 2 years of age. Therefore, a single positive result for anti-HHV-6 IgG may not be able to differentiate recent infection from remote exposure. The most commonly used molecular test for the laboratory diagnosis of HHV-6 is NAAT and at least one multiplex test platform for this is FDA-cleared; some of these tests differentiate variants A and B (Table 53). However, qualitative NAAT does not differentiate replicating from latent virus. HHV-6 DNA quantification may be useful in this regard, as well as in monitoring response to antiviral therapy. HHV-6 may be shed intermittently by healthy and immunocompromised hosts. Therefore, detection of HHV-6 in blood, body fluids, or even tissue does not definitively establish a diagnosis of disease caused by HHV-6. Chromosomally integrated HHV-6, which results in high HHV-6 levels in virtually all clinical specimens, may lead to an erroneous diagnosis of active infection. HHV-6 can be cultured from peripheral blood mononuclear cells (and other clinical specimens) [269]. However, viral isolation is labor-intensive, taking up to 21 days. The detection time can be shortened to 1–3 days with the use of shell vial culture assay. In addition to a long processing time, culture-based assays suffer from poor sensitivity and do not differentiate between variants A and B. If tissue biopsy is performed, HHV-6 antigens can be targeted by immunohistochemical or in situ hybridization tests in formalin-fixed, paraffin-embedded tissues.

G. Parvovirus (Erythrovirus) B19 ::: XIV. VIRAL SYNDROMES:
Parvovirus B19 is associated with a variety of clinical syndromes including erythema infectiosum (ie, "slapped-cheek" rash or "gloves-and-socks" syndrome) or arthralgia/arthritis in immunocompetent individuals, transient aplastic crisis in patients with hemoglobinopathies or who are otherwise immunosuppressed, and congenital infection and possibly fetal death (eg, hydrops fetalis) occurring in nonimmune women who acquire the virus during pregnancy. Disease is often biphasic beginning as a self-resolving, nonspecific febrile illness, followed by onset of rash and/or arthralgia approximately 1 week later. Importantly, the classic rash is immunologically mediated, as its appearance corresponds with development of an IgM antibody response to the virus. Serologic testing for the presence of IgM- and/or IgG-class antibodies to parvovirus B19 is the recommended diagnostic testing method for evaluation of a parvovirus B19 infection (Table

54). IgM-class antibodies to the virus are detectable within 10–12 days postinfection, with IgG detectable by 2 weeks [270–272]. Notably, approximately 90% of patients presenting with erythema infectiosum have detectable IgM antibodies to parvovirus B19 at the time of presentation [272]. Antibodies to parvovirus B19 reach peak titers within 1 month, and while the presence of IgM-class antibodies suggests recent infection, they can persist for months. The presence of IgG antibodies alone is indicative of past exposure; these may remain detectable for life and are thought to provide lasting immunity to reinfection. Serologic testing for parvovirus B19 remains the recommended methodology for evaluation of pregnant women with possible exposure or infection; positive results for both IgM and IgG antibodies to parvovirus B19 suggest infection within the last 3 months and a possible risk of infection to the fetus. Importantly, serologic tests may be negative in an immunocompromised host, despite prior exposure to the virus.

Parvovirus B19 NAATs may provide improved sensitivity over serologic methods in patients presenting with transient aplastic crisis or chronic anemia. Despite the lack of FDA-cleared molecular assays for parvovirus B19, NAAT is the preferred noninvasive technique for laboratory diagnosis of parvovirus B19-related anemia in immunosuppressed individuals, including solid organ transplant recipients. An important caveat regarding NAAT for diagnosis of parvovirus B19-related anemia is that parvovirus B19 DNA has been anecdotally detected for extended periods in serum, even in healthy individuals [273]. The presence of giant pronormoblasts in bone marrow is suggestive of parvovirus B19 infection, although such cells are not always detected.

H. Measles (Rubeola) Virus ::: XIV. VIRAL SYNDROMES:

Although endemic measles was proclaimed eliminated in the United States in 2000 as a result of high vaccination rates and vaccine efficacy (~97% following 2 doses), travel-associated cases (and spread among unvaccinated individuals) continue to occur (www.cdc.gov/measles/vaccination.html). Immunity to measles is indicated by the presence of IgG-class antibodies to the virus. While diagnosis of recent (acute) measles infection can be made on clinical grounds, supportive laboratory findings include a positive antimeasles IgM result. IgM antibodies are often positive by the time the rash appears, but up to 20% of patients may be serologically negative within the 72 hours after rash onset. Therefore, in suspected measles cases, initially seronegative cases during the acute stage, a second specimen collected 72 hours after rash onset should be collected and tested for antimeasles IgM to document seroconversion. IgM antibodies to measles may be detectable for a month or longer following disease onset and may also be positive in recently vaccinated individuals. A serologic diagnosis of acute measles may be established by demonstrating seroconversion of antimeasles IgG antibodies or a 4-fold rise in IgG titers between acute (collected at the time of rash onset) and convalescent (collected 10–30 days later) specimens (Table 55). Notably however, quantitative or semi-quantitative testing for antimeasles antibodies (ie, determining a titer) is no longer routinely available in local or reference laboratories. Measles virus can be isolated by culture or detected by NAAT from throat, nasal or nasopharyngeal swabs, or urine collected soon after rash onset; such testing is typically limited to public health laboratories [274].

Infrequently, measles infection may lead to development of subacute sclerosing panencephalitis (SSPE) later in life. Measurement of antibodies to measles in CSF is recommended in suspected cases of SSPE. Importantly, intrathecal antibody synthesis of these antibodies should be confirmed by ruling out introduction of antimeasles antibodies into the CSF via blood contamination (eg, during a traumatic lumbar puncture) or defective blood–brain barrier permeability.

I. Mumps Virus ::: XIV. VIRAL SYNDROMES:

Similar to measles, mumps is considered eliminated in the United States, though travel-associated cases among unvaccinated individuals continue to occur, and while effective, the mumps vaccine has a protective rate of approximately 88% following administration of the 2 doses (www.cdc.gov/mumps/vaccination.html). Immunity to mumps is suggested by the presence of antimumps IgG-class antibodies. While mumps infection presents with classic symptoms (eg, parotitis), diagnosis of infection can be supported by a positive serologic test for antimumps IgM antibodies and/or seroconversion or a 4-fold rise of mumps IgG antibody levels between acute and convalescent phase sera (Table 56). Ideally, acute phase sera should be collected immediately upon suspicion of mumps virus infection and/or symptom onset and convalescent sera collected approximately 5–10 days thereafter. IgM antibodies to mumps typically become detectable during the first few days of illness, peak approximately 1 week after

onset, and may remain detectable for a few months. As with serologic testing for measles, quantitative or semi-quantitative (ie, determining a titer) testing for mumps IgG-class antibodies is no longer routinely available in local or reference laboratories.
Notably, previously immunized patients who are subsequently infected with mumps may not develop a detectable IgM response to the virus. For such individuals, confirmation of mumps infection requires isolation of the virus itself or detection of viral RNA; these tests are largely limited to public health laboratories and the CDC. The preferred specimen source for culture and/or NAAT is an oral or buccal swab around the affected parotid gland and Stensen duct [275]. Mumps virus RNA may be detected prior to onset of parotitis until 5–9 days after symptom onset. Unlike for measles, urine samples are not considered as sensitive for mumps culture or NAAT, as the virus is often not detected in this specimen source until at least 4 days following symptom onset.

J. Rubella Virus ::: XIV. VIRAL SYNDROMES:
Rubella (German measles or 3-day measles) was officially proclaimed eliminated from the United States in 2004, largely due to intense vaccination efforts; with <10 cases reported per year, these are often travel associated and sporadic. Serologic testing for detection of antirubella antibodies can be used to establish immunity or to provide laboratory-based evidence for rubella infection (Table 57). The presence of IgG antibodies to rubella virus in an asymptomatic individual indicates lifelong immunity to infection. Acute rubella infection can be serologically confirmed by documenting seroconversion to IgM and/or IgG positivity or a 4-fold rise in antirubella IgG titers between acute and convalescent serum specimens. As with measles and mumps serologic assays, however, assays providing quantitative titers for antibodies to rubella are not commonly offered at local or reference laboratories.
Only approximately 50% of patients are positive for IgM antibodies to rubella at the time of rash onset, which emphasizes the importance of collecting a convalescent sample. Acute phase serum should be collected upon patient presentation and again 14–21 days (minimum of 7) days later. Due to the rarity of rubella in the United States and thus the low pretest probability of infection, serologic evaluation should only be performed in patients with appropriate exposure risks and a clinical presentation highly suggestive of acute rubella; in patients not meeting these criteria, positive rubella IgM results should be interpreted with caution as they may be falsely positive. Congenital rubella syndrome can be diagnosed by the presence of IgM-class antibodies to rubella in a neonate, alongside symptoms consistent with congenital rubella syndrome, appropriate exposure history of the mother, and lack of maternal protective immunity. NAAT for detection of rubella RNA can be performed on throat or nasal swabs and urine, though such testing is largely limited to public health laboratories and/or the CDC. Specimens for NAAT should be collected within 7 days of presentation to enhance sensitivity.

K. BK Virus ::: XIV. VIRAL SYNDROMES:
BK virus is a polyomavirus that may cause allograft nephropathy in renal transplant recipients and hemorrhagic cystitis, especially in bone marrow transplant patients. A definitive diagnosis of these conditions requires renal allograft biopsy with in situ hybridization for BK virus.
Detection of BK virus by NAAT in plasma may provide an early indication of allograft nephropathy, although there are currently no FDA-cleared NAATs (Table 58) [276]. Urine cytology or quantitative NAAT may be used as a screening test, and if positive, may be followed by BK viral load testing of plasma, which has a higher clinical specificity. As there are no FDA-cleared quantitative NAATs available for monitoring BK viral loads, each institution must establish a threshold for identifying patients at highest risk of BK virus–associated nephropathy. Urine NAATs for BK virus may be more sensitive than detection of decoy cells (virus-infected cells shed from the tubules or urinary tract epithelium) using urine cytology, as BK virus DNA is typically detectable earlier in the urine than are decoy cells. However, shedding of BK virus in urine is common. Therefore, if used as a screening test, only high levels (ie, above a laboratory-established threshold that correlates with disease) should be considered significant. Urine testing for BK virus places the laboratory at risk for specimen cross-contamination, as extremely high levels of virus in the urine may lead to carryover between specimens and, potentially, false-positive results.

L. JC Virus ::: XIV. VIRAL SYNDROMES:
JC virus is the etiologic agent of progressive multifocal leukoencephalopathy (PML), which is a fatal, demyelinating disease of the CNS that occurs in immunocompromised hosts. Histologic examination of brain biopsy tissue may reveal characteristic pathologic changes; however, in situ

hybridization for JC virus may be required to confirm the diagnosis. Detection of JC virus DNA in CSF specimens by NAAT has largely replaced the need for tissue biopsy for laboratory diagnosis of PML (Table 59). A serologic test (STRATIFY JCV) is now FDA-cleared for screening patients who are considering treatment with certain immunomodulating therapies (eg, natalizumab). A positive result by this test is indicative of prior exposure to JCV, and potentially elevated risk of developing PML, if initiating treatment with the immunomodulating drug natalizumab.

M. Dengue Virus ::: XIV. VIRAL SYNDROMES:
Dengue virus (DENV) is a flavivirus transmitted by Aedes spp mosquitos and is most often associated with a febrile illness in travelers returning from endemic regions (eg, Caribbean, South and Central America, Asia). Diagnosis of DENV infection is most often established by serologic methods for detection of IgM- and/or IgG-class antibodies to the virus or detection of the DENV nonstructural protein 1 (NS1) antigen (Table 60). In cases of primary infection, IgM-class antibodies to DENV are detectable as early as 3–5 days after symptom onset and remain detectable for 2–3 months, whereas IgG antibodies to the virus appear 10–12 days after onset and are detectable for months to years [277]. Notably, in secondary or repeat DENV infection, IgM antibodies may not be detectable. An initially negative serologic profile for DENV in a patient for whom dengue fever is strongly suspected should be followed up with repeat serologic evaluation on a serum specimen collected 7–10 days after disease onset. Seroconversion to either anti-DENV IgM and/or IgG seropositivity is strongly suggestive of recent infection. However, due to the similar antigenic profiles between members of the Flavivirus genus, false-positive results for antibodies to DENV may occur in patients with a prior flavivirus infection (eg, West Nile virus, St Louis encephalitis virus, or Zika virus). Plaque reduction neutralization tests (PRNTs) are considered the reference standard for detection of antibodies to arthropod-borne viruses (arboviruses) and provide improved specificity over commercial serologic assays; however, due to the complexity of testing, PRNT is currently only available at select public health laboratories and the CDC.
Following infection with DENV, patients may be viremic for 4–6 days after symptom onset. Though viral isolation is possible during this timeframe, it is not routinely performed in clinical laboratories [278]. Detection of DENV RNA by NAAT is preferred for acutely ill patients. Recently, detection of the DENV NS1 antigen, which is secreted from infected host cells as early as 1 day after symptom onset and up to 10 days thereafter, has become an acceptable alternative to NAAT for diagnosis of acute DENV infection.

N. Hepatitis A and E Viruses ::: XIV. VIRAL SYNDROMES:
Diagnosis of acute hepatitis A virus (HAV) infection is confirmed by detecting HAV IgM antibody (Table 61). However, false-positive HAV IgM antibody results can occur due to low positive predictive value of assays used in population with low prevalence of acute hepatitis A [279]. The presence of HAV IgG antibody indicates either past or resolved hepatitis A infection or immunity to this viral infection from vaccination. Alternatively, the same hepatitis A state can be deduced by the presence of combined HAV IgG and IgM total antibodies in an asymptomatic patient with normal liver tests and/or absence of HAV IgM antibody.
Hepatitis E is usually a foodborne illness in developing countries due to ingestion of hepatitis E virus (HEV) transmitted in contaminated food and water. However, such infection in developed countries may be encountered in return travelers (acute hepatitis E) or organ transplant recipients (acute or chronic) [280]. Because presentation of acute hepatitis A and E are indistinguishable clinically from one another, diagnosis of the latter is made usually by presence of HEV IgM antibody (appearing by 4–6 weeks after exposure and lasting for 2–4 months) and absence of HAV IgM antibody in serum or plasma. HEV IgG antibody is detectable in serum and plasma usually by 4 weeks after clinical presentation. However, with delayed humoral response in organ transplant recipients who are immunosuppressed from antirejection therapy and suspected to have acute hepatitis E, diagnosis may need to be made with molecular assays for detection of HEV RNA in serum or plasma. Individuals with ≥3 months of HEV viremia are considered to have chronic hepatitis E, and quantification of HEV RNA in serum or plasma can be used to monitor disease progression and response to antiviral therapy.

O. Hepatitis B, D, and C Viruses ::: XIV. VIRAL SYNDROMES:
Hepatitis B surface antigen (HBsAg) may be detected in the presence of acute or chronic hepatitis B virus (HBV) infection [281]; it indicates that the person is infectious. In acute infection, its appearance predates clinical symptoms by 4 weeks and it remains detectable for 1–6 weeks. The

tests for detecting hepatitis B and D disease are primarily serologic and molecular (Table 62). Care providers should check with the laboratory on the minimum volumes of blood needed, as some molecular platforms require more blood than others.

The presence of hepatitis B surface antibody (HBsAb) indicates recovery from and immunity to HBV infection, as a result of either natural infection or vaccination. In most patients with self-limited acute HBV infection, HBsAg and HBsAb are not detectable simultaneously in serum or plasma.

Hepatitis B core (HBc) IgM antibody appears during acute or recent HBV infection and remains detectable for about 6 months. A serologic "window" occurs when HBsAg disappears and HBsAb is undetectable. During this "window" period, infection can be diagnosed by detecting HBc IgM antibody.

HBc total antibodies appear at the onset of symptoms of acute hepatitis B infection and persist for life. Their presence indicates acute (positive HBc IgM antibodies), recent (both HBc IgM and HBc total antibodies), or previous (positive HBc total antibodies but negative HBc IgM antibody) HBV infection. There is currently no commercially available test for HBc IgG antibody in serum or plasma.

A chronic HBV carrier state is defined by persistence of HBsAg for at least 6 months. In patients with chronic hepatitis B, the presence of hepatitis B e antigen (HBeAg) in serum or plasma is a marker of high viral replication levels in the liver. Loss of HBeAg and emergence of antibody to HBeAg (ie, HBe antibody) is usually associated with improvement of underlying hepatitis and a reduction in the risk of hepatocellular carcinoma and cirrhosis. Alternatively, disappearance of HBeAg may denote the emergence of a precore mutant virus; high concentrations of HBsAg and HBV DNA, in the absence of HBeAg and presence of HBe antibody, suggest the presence of a HBV precore mutant virus. Hepatitis B viral DNA is present in serum or plasma in acute and chronic hepatitis B infection [282]. Quantification of HBV DNA in serum or plasma may be included in the initial evaluation and management of chronic hepatitis B infection, especially when the serologic test results are inconclusive or when deciding treatment initiation and monitoring a patient's response to therapy. Other molecular laboratory tests used in the diagnosis and management of chronic hepatitis B infection have been reviewed and include assays for determining viral genotype, detection of genotypic drug resistance mutations, and core promoter/precore mutations [282].

Detection of HBs antibody in the absence of HBc total antibodies distinguishes vaccine-derived immunity from immunity acquired by natural infection (in which both HBs antibody and HBc total antibodies are present). Current commercially available assays for detecting HBs antibody yield positive results (qualitative) when antibody levels are ≥10 mIU/mL (or ≥10 IU/L) in serum or plasma, indicating postvaccination immunity (protective antibody level). Quantitative HBs antibody results are used to monitor adequacy of hepatitis B immunoglobulin therapy in liver transplant recipients receiving such therapy during the posttransplant period.

In acute hepatitis D superinfection of a patient with known chronic hepatitis B, hepatitis D virus (HDV) antigen, HDV IgM, and total antibodies are present (Table 62). In acute hepatitis B and D coinfection, the same serologic markers (ie, HDV antigen, HDV IgM, and total antibodies) are present, along with HBc IgM antibody.

The diagnosis of hepatitis C virus (HCV) infection usually begins with a screening test for HCV IgG antibody in serum or plasma immunoassays. Antibody may not be detectable until 6–10 weeks after the onset of clinical illness. Individuals with negative HCV antibody screening test results do not need further testing for hepatitis C (Table 63), except in immunocompromised individuals (in whom development of HCV IgG antibody may be delayed for up to 6 months after exposure) or those with suspected acute HCV infection. Those with positive screening HCV IgG antibody test results should undergo confirmatory or supplemental testing for HCV RNA by molecular test methods. Signal-to-cutoff ratios (calculated by dividing the optical density value of the sample tested by the optical density value of the assay cutoff for that run) are an alternative to supplemental testing (http://www.cdc.gov/hepatitis/HCV/LabTesting.htm). Supplemental HCV IgG antibody assays can confirm the presence of HCV antibodies in patients with positive HCV IgG antibody screening test results, but none of these assays are currently FDA-approved for clinical use in the United States. According to the latest recommendations from the CDC [283], all individuals born during 1945–1965 should be screened at least once for evidence of HCV infection.

Hepatitis C virus RNA can be detected by NAATs soon after infection as well as in chronic infection. NAATs for HCV can be performed qualitatively or quantitatively (by RT-PCR or transcription-mediated amplification methods). Highly sensitive molecular assays for

quantification of HCV RNA in serum or plasma (limit of detection of ≤25 IU/mL) are necessary to monitor patients' virologic response and to determine cure (ie, sustained virologic response) from antiviral therapy. Determination of HCV genotype and subtypes (ie, 1–6 and 1a vs 1b) is used to guide the choice and duration of antiviral therapy and predict the likelihood of response to therapy, as different genotypes and subtypes varying in virologic response to current treatment regimens and in likelihood of antiviral resistance before or during direct-acting antiviral (DAA) treatment. Pretreatment testing for HCV genome-specific resistance-associated substitutions (RASs) by conventional (Sanger) or next-generation sequencing assay methods is recommended by the FDA and/or current clinical practice guideline (https://www.hcvguidelines.org/evaluate/resistance) prior to initiating certain DAA therapy combinations for infection due to certain HCV genotypes: (1) HCV NS3 RAS for simeprevir in genotype 1 infection, and (2) HCV NS5A RAS for elbasvir-grazoprevir or ledipasvir/sofosbuvir in genotype 1a infection, and daclatasvir/sofosbuvir or velpatasvir/sofosbuvir in genotype 3 infection. Per recent recommendations from the FDA (https://www.fda.gov/Drugs/DrugSafety/ucm522932.htm), all patients prior to initiating DAA therapy should be screened for evidence of prior or current HBV infection (positive for HBc total antibodies and/or HBsAg), so that affected patients can be monitored and managed appropriately for reactivation of HBV during and after DAA therapy.
A human genomic polymorphism interleukin 28B (IL-28B) genotype CC (within an interferon-γ promoter region on human chromosome 9) is associated with good likelihood of spontaneous resolution of HCV infection in acutely infected individuals as well as high probability of sustained viral response in those receiving interferon-based combination therapy for chronic HCV infection. Now with interferon-based therapy no longer in use for chronically HCV-infected individuals, IL-28B genotype testing is used mainly to predict likelihood of spontaneous resolution of acute HCV infection.

P. Enterovirus and Parechovirus ::: XIV. VIRAL SYNDROMES:
Enteroviruses are a large group of viral pathogens that may cause disease ranging from mild respiratory infection, to paralysis or severe CNS infection. NAAT of CSF is more sensitive than viral culture for the diagnosis of enteroviral CNS infection (Table 64). Plasma or serum is useful for diagnosis of sepsis syndrome in a newborn due to enterovirus, but testing is less reliable beyond the newborn period. In the right clinical scenario, detection of enterovirus from throat or stool specimens may provide circumstantial evidence of CNS infection; however, if this is performed, it should be accompanied by NAAT testing of CSF.
Serologic evaluation for enteroviruses requires assessment of acute and convalescent titers, due to the high seroprevalence in the population. Therefore, serology is typically not useful in clinical practice, with the exception of determining whether a patient with myocarditis has had exposure to enteroviruses (eg, Coxsackie B virus).
Parechoviruses have clinical presentations similar to enteroviruses, but are classified as a different genus and require a specific NAAT for detection (laboratory validated only, except for one current multiplex assay that is FDA-cleared).

Q. Respiratory Syncytial Virus ::: XIV. VIRAL SYNDROMES:
Respiratory syncytial virus causes bronchiolitis and/or pneumonia and is most common in infants and young children, although it can cause respiratory illness in adults and severe disease in immunocompromised hosts. NAAT testing has become the diagnostic method of choice, and the preferred specimen types include a nasopharyngeal swab or BAL fluid, if the patient has evidence of lower respiratory tract infection (Table 65). Several FDA-cleared NAAT platforms exist. Although RSV can be recovered in routine viral culture, this approach is time-consuming and cytopathic effect may not be observed for up to 2 weeks.
Serology is not recommended as a diagnostic method in patients with suspected RSV infection. The seroprevalence to RSV is high, and the presence of IgG-class antibodies generally indicates past exposure and immunity.

R. Influenza Virus Infection ::: XIV. VIRAL SYNDROMES:
Rapid diagnosis of influenza virus infection (≤48 hours following the onset of symptoms) is needed to facilitate early administration of antiviral therapy. The virus may be rapidly detected by NAAT or direct antigen detection from a nasopharyngeal swab (Table 66). Sensitivity is higher for NAAT than rapid antigen detection. Rapid screening tests may perform poorly during influenza season (especially for detection of pandemic H1N1 and swine-associated H3N2 strains), and negative tests should be confirmed by NAAT or culture prior to ruling out influenza infection.

During seasons of low prevalence of influenza, false-positive tests are more likely to occur when using rapid antigen tests. The performance of influenza assays, including NAAT and rapid antigen tests, varies depending on the assay and the circulating strains. NAAT is now considered the gold standard for detection of influenza virus in clinical samples. Several FDA-cleared NAAT platforms exist.
Influenza virus can be recovered in routine viral cell culture, but confirmation is needed, typically through the use of hemadsorption and/or hemagglutination techniques. Serologic testing is not useful for the routine diagnosis of influenza due to high rates of vaccination and/or prior exposure.

S. West Nile Virus ::: XIV. VIRAL SYNDROMES:
West Nile virus (WNV), alongside other endemic arboviruses including St Louis encephalitis, Lacrosse encephalitis, and California encephalitis viruses, can cause CNS infections. Laboratory diagnosis of WNV, and most other arboviruses, is typically accomplished by detecting virus-specific IgM- and/or IgG-class antibodies in serum and/or CSF [284] (Table 67). IgM antibodies to WNV are detectable 3–8 days after symptom onset and often taper off 2–3 months later, though seropersistence in serum for up to 12 months has been documented. Seroconversion to anti-WNV IgM and/or IgG positivity between acute and convalescent sera (collected 7–10 days apart) is strongly suggestive of a recent WNV infection. The presence of anti-WNV IgG alone at the time of presentation is indicative of prior WNV infection, and evaluation for an alternative etiology is recommended. Serologic diagnosis of WNV CNS infection may be established by detection of IgM antibodies to WNV in CSF as antibodies in this class do not naturally cross the blood–brain barrier. However, introduction of blood into the CSF during a traumatic lumbar puncture or defective permeability of the blood–brain barrier may lead to falsely elevated IgM levels in the CSF. False-positive results for both anti-WNV IgM and IgG antibodies may occur in patients who have been vaccinated against yellow fever virus or following natural infection with other flaviviruses (eg, dengue, St Louis encephalitis viruses). To rule out cross-reactivity, it is recommended that specimens reactive for WNV antibodies be tested by PRNT.
Detection of WNV RNA by NAAT in serum and CSF is associated with higher sensitivity in immunosuppressed patients due to the delayed immune response and thus prolonged WNV viremia in this population. Viral culture, while possible, is insensitive and not routinely offered at local or reference laboratories.

T. Adenovirus ::: XIV. VIRAL SYNDROMES:
In otherwise healthy individuals, adenoviruses may cause mild, self-limiting respiratory illness or conjunctivitis, with most cases being diagnosed on clinical grounds. Occasionally, adenovirus infections in immunocompetent hosts can result in death, especially in children with asthma. In immunocompromised patients, adenoviruses may cause pneumonia, disseminated infection, gastroenteritis, hemorrhagic cystitis, meningoencephalitis, or hepatitis.
The diagnosis of adenoviral infections is typically made using NAAT, viral culture, and/or histopathology (Table 68). Viral culture has a long turnaround time (~5 to 7 days), but this can be reduced by using shell vial technology. Plasma viral load (assessed by quantitative NAAT) may be useful as a marker for preemptive therapy, to diagnose adenovirus-associated signs and symptoms, and to monitor response to antiviral therapy in some immunocompromised populations.

U. Rabies Virus ::: XIV. VIRAL SYNDROMES:
Rabies virus infects the CNS and is most often transmitted through the bite of a rabid animal. Diagnostic testing for rabies is not offered through most hospital or reference laboratories; therefore, consultation with a local public health laboratory or the CDC should be performed immediately in suspected rabies cases.
No single test is sufficient to diagnose rabies antemortem (Table 69). NAATs and viral isolation can be performed on saliva, immunohistochemistry may be performed on skin biopsies at the nape of the neck for detection of rabies antigen in the cutaneous nerves, and antirabies antibody testing is available for serum and CSF specimens. Postmortem histopathology of brain biopsies in patients with rabies are notable for mononuclear infiltration, perivascular cuffing of lymphocytes, lymphocytic foci, and Negri bodies. Serologic testing may be used to document postvaccination seroconversion, if there is significant deviation from a prophylaxis schedule.

V. Lymphocytic Choriomeningitis Virus ::: XIV. VIRAL SYNDROMES:

Lymphocytic choriomeningitis virus (LCMV) is a rodent-borne virus that can cause meningoencephalitis and may be life-threatening in immunosuppressed persons. Serologic testing is the mainstay of diagnosis for LCMV infection, and is typically established by demonstrating a 4-fold or greater increase in IgG-class antibody titers between acute and convalescent phase serum samples, or by detection of anti-LCMV IgM antibodies (Table 70). Detection of antibodies in the CSF may indicate CNS infection; however, it may also be observed if the CSF fluid becomes contaminated with blood during collection, or if there is transfer of antibodies across the blood–brain barrier. NAAT can also be used to diagnose LCMV infection, but is limited to select public health laboratories.

W. Human Coronavirus ::: XIV. VIRAL SYNDROMES:
The coronaviruses are host specific and can infect a variety of animals as well as humans. Four distinct genera have been described with human pathogens belonging to the genera alphacoronavirus (229E and NL63) and betacoronavirus, lineage A (OC43 and HKU1) lineage B (severe acute respiratory syndrome [SARS] coronavirus), and lineage C (Middle East respiratory syndrome [MERS] coronavirus).
Human coronaviruses 229E, NL63, OC43, and HKU1 are associated with the common cold with symptoms of rhinorrhea, congestion, sore throat, sneezing, and cough and may present with fever. In children, the viruses have also caused exacerbation of asthma and otitis media.
Respiratory secretions or nasopharyngeal swabs placed in appropriate VTM are the specimens of choice. Diagnostic tests include NAATs, which are now common in commercial respiratory panels.
Suspected cases of SARS coronavirus and MERS coronavirus require immediate notification to the laboratory. Guidance for testing can be found at www.cdc.gov/sars/index.html and www.cdc.gov/coronavirus/MERS/index.html.

Parainfluenza ::: XIV. VIRAL SYNDROMES:
Parainfluenza viruses are a major cause of croup (laryngotracheobronchitis), bronchiolitis, and pneumonia as well as upper respiratory tract infections. Of the 4 antigenically distinct types, types 1 and 2 are most commonly associated with croup syndrome, while type 3 is associated most commonly with bronchiolitis and pneumonia. Parainfluenza virus infections account for up to 11% of all hospitalizations in children <5 years old [285].
Respiratory secretions or nasopharyngeal swabs placed in appropriate VTM are the specimens of choice. Diagnostic tests include culture, which may take 4–7 days for detection, and NAATs, which are now common in commercial respiratory panels.

Y. Human Metapneumovirus ::: XIV. VIRAL SYNDROMES:
Human metapneumovirus has been shown to cause acute respiratory tract disease in people of all ages. The virus has been associated with cases of bronchiolitis in infants as well as pneumonia, exacerbations of asthma and croup, and upper respiratory infections with concomitant otitis media in children. Most commonly, children present with mild to moderate symptoms. Infections with human metapneumovirus associated with exacerbations of chronic obstructive pulmonary disease pneumonia have been detailed in adults. When diagnostic tests are required, the specimens of choice are respiratory secretions or nasopharyngeal swabs placed in VTM. Diagnostic tests include immunofluorescent assays and NAATs, which are now available in several commercial respiratory panels.

Z. Zika Virus ::: XIV. VIRAL SYNDROMES:
Zika virus (ZIKV), a member of the Flavivirus genus and transmitted by Aedes spp mosquitos, has been causally linked to congenital birth defects, including microcephaly [286]. Diagnostic tests available for ZIKV include NAATs for viral RNA, serologic evaluation for IgM antibodies to the virus, and PRNTs, considered the reference standard for detection of neutralizing antibodies to arboviruses (Table 71). Selection between these methodologies (ie, NAAT vs serology) is primarily dependent on when the patient presents in relation to symptom onset or last possible exposure to ZIKV [287]. Currently, the CDC recommends molecular testing by NAAT on serum and urine in symptomatic patients and pregnant women with illness duration of 14 days or less. While a positive NAAT result for ZIKV is diagnostic for infection, a negative result does not exclude infection as viremia or viruria may have passed by the time the specimen was collected. Patients negative by NAAT, particularly pregnant women, for whom ZIKV infection remains in the differential, should be evaluated using a serologic assay for antibodies to the virus. The most current guidelines and recommendations regarding evaluation and testing for ZIKV can be found

on the CDC website (https://www.cdc.gov/zika/hc-providers/index.html). False-positive ZIKV IgM serologic results may occur in patients with a prior or current infection with a closely related flavivirus, including WNV or DENV. Therefore, clinical decisions regarding patient management should not be based on a reactive ZIKV IgM serologic result alone. Confirmatory PRNTs for ZIKV neutralizing antibodies should be performed for all samples reactive by an anti-ZIKV IgM serologic assay. Finally, due to co-circulation of DENV and Chikungunya viruses in regions where ZIKV is endemic, and the often-times similar disease manifestation among these arboviruses, concurrent evaluation for DENV and Chikungunya virus should be considered.

A. Babesia and Malaria ::: XV. BLOOD AND TISSUE PARASITE INFECTIONS:

Babesiosis is caused primarily by Babesia microti in the United States and Babesia divergens in Europe [293]. More recently, a small number of infections occurring in California and Washington have been attributed to Babesia duncani, while B. divergens–like organisms including the MO-1 strain have been detected in patients residing in Missouri, Kentucky, Washington, and Arkansas [293]. Human malaria is caused by 4 Plasmodium spp: P. falciparum, P. vivax, P. ovale, and P. malariae [287, 288]. The simian parasite Plasmodium knowlesi has also been reported to cause a significant portion of human cases in parts of Southeast Asia [293]. Table 73 summarizes the laboratory tests available for these agents.

The gold standard method for diagnosis of both malaria and babesiosis is microscopic examination of Giemsa-stained thick and thin blood films [293, 294]. Although this method requires a minimum amount of resources (staining materials and high-quality, well-maintained microscopes), skilled and experienced technologists must be available to obtain maximum accuracy and efficiency. Because both babesiosis and malaria are serious infections that can progress to fatal outcomes if not diagnosed and treated accurately, it is necessary for healthcare facilities to have ready access to rapid accurate laboratory testing [293]. Samples should be obtained from fresh capillary or ethylenediaminetetraacetic acid (EDTA) venous blood and slides prepared and read immediately.

Thick blood films are essentially lysed concentrates which allow rapid detection of the presence of parasites consistent with either Plasmodium or Babesia but may not allow for differentiation of the 2 organisms. The thick film is made using 2–3 drops of blood that have been “laked” (lysed) by placement into a hypotonic staining solution. This releases the intracellular parasites and allows for examination of multiple (20–30) layers of blood simultaneously. For this reason, it is the most sensitive method for microscopic screening and allows detection of very low levels of parasitemia (<0.001% of red blood cells [RBCs] infected) [293]. Use of the “scratch method” allows for improved adherence of the thick film to the slide and facilitates rapid examination (ie, it can be examined as soon as the blood is visibly dry) [294]. In contrast to thick films, thin films are prepared like a hematology peripheral smear and are fixed in ethanol before staining. Fixation retains the structure of the RBCs and intraerythrocytic parasites and provides ideal morphology for Plasmodium spp identification. It also allows for optimal evaluation and differentiation of Plasmodium from Babesia parasites, although the different Babesia spp cannot be distinguished from one another by morphology alone. Staining is best performed with Giemsa at a pH of 7.2 to highlight the microscopic features of the parasites. Wright-Giemsa and rapid Field stains are also acceptable. The CDC provides additional guidelines for inactivating hemorrhagic fever viruses such as Ebola in clinical specimens (https://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimen-management.html).

Both thick and thin films should be screened manually, since automated hematology analyzers may fail to detect Plasmodium and Babesia spp parasites [293]. The slides should first be screened at low power using the 10× objective for identification of microfilariae, followed by examination under oil immersion [290, 291, 293, 294]. The laboratorian should examine a minimum of 100 microscopic fields using the 100× objective on the thick and thin films before reporting a specimen as negative. Additional fields (at least 300) should be examined for patients without previous Plasmodium exposure since they may be symptomatic at lower parasite levels [294]. It is important to remember that Babesia and Plasmodium may at times be indistinguishable on blood films and that both can be transmitted by transfusion, so each can occur in atypical clinical settings. Clinical and epidemiologic information must be considered and additional testing may be required.

If parasites are identified and the laboratory does not have expertise for species identification, then a preliminary diagnosis of “Plasmodium or Babesia parasites” should be made, followed by confirmatory testing at a reference or public health laboratory. The CDC provides rapid telediagnostic services for this purpose (http://www.cdc.gov/dpdx/contact.html). While awaiting

confirmatory testing, the primary laboratory should relay the message to the clinical team that the deadly parasite P. falciparum cannot be excluded from consideration. Repeat blood samples (≥3 specimens drawn 12–24 hours apart, ideally during febrile episodes) are indicated if the initial film is negative and malaria or babesiosis is strongly suspected.

When Plasmodium spp are identified, one can enumerate the number of infected RBCs and divide by the total number of RBCs counted to arrive at the percentage of parasitemia. This is best determined by using the thin film. Quantification can also be performed using the thick film, but this method is less precise. Quantification is used to guide initial treatment decisions and to follow a patient's response to antimalarial treatment [293].

An alternative to Giemsa-stained blood films for morphologic examination is the quantitative buffy coat (QBC) method [293]. This test detects fluorescently stained parasites within RBCs and requires specialized equipment. It acquires maximum efficiency for the laboratory if multiple specimens are being processed at the same time, which is seldom the case in US laboratories. In addition, it requires preparation of a thin blood smear if a QBC sample is positive, since specific identification and rate of parasitemia will still need to be determined by the latter method [293]. For these reasons, the QBC method is seldom used in the United States at this time.

Although morphologic examination is the conventional method for diagnosis of malaria, it requires considerable time and expertise. Malaria RDTs provide cost-effective, rapid alternatives and can be used for screening when qualified technologists are not available [288]. These methods are rapid immunochromatographic tests using dipstick, card, or cassette formats in which a nitrocellulose membrane with bound parasite antigens are incorporated. The most commonly used antigens are Plasmodium lactate dehydrogenase, Plasmodium aldolase, and P. falciparum histidine-rich protein 2. There are a number of commercially available options, although the BinaxNow Malaria is currently the only test approved by the FDA. Depending on the number of antigens employed, RDTs may detect to the genus level, species level (most commonly P. falciparum), or both. In general, RDTs are somewhat less sensitive than thick blood films and may be falsely negative in cases with very low rates of parasitemia and non-falciparum infection [292]. The performance characteristics of the commercially available assays vary widely; the WHO provides several useful publications on the performance and selection of available malaria RDTs [292]. Given the lower sensitivity, positive RDTs should be confirmed by examination of thick and thin blood films, ideally within 12–24 hours of patient presentation. Blood film examination is also necessary for positive cases to confirm the species present and calculate the degree of parasitemia [295]. In the United States, Canada, and Europe, RDTs are primarily used for initial screening in settings where reliable blood films are not be readily available (evening shift, small community laboratories) or when the clinical situation is critical and an immediate diagnosis is required (stat laboratory in the emergency department). Such RDT testing should be followed as soon as possible by good-quality thick and thin blood film examination. It is important to note that RDTs may be falsely positive for several days after eradication of intact parasites since antigens may still be detected. Therefore, the assay should not be used to follow patients after adequate therapy has been given.

Serology plays little role in diagnosis of acute babesiosis and malaria, since antibodies may not appear early in infection and titers may be too low to determine the status of infection. The primary use of antibody detection is for epidemiologic studies and as evidence of previous or relapsing infection. Serologic testing is also used for blood donor screening. IFA is the most readily available commercial assay for Babesia; IgM titers ≥1:16 and IgG titers ≥1:1024 indicate acute infection, as does a 4-fold rise in titer. IgG titers of 1:64–1:512 with negative IgM and no titer rises in serial specimens suggest previous infection or exposure. There is insufficient evidence for use in diagnosis of B. divergens, B. duncani, or MO-1 infections. Serology for Plasmodium spp is available through the CDC.

Rapid NAATs have recently been developed for malaria and babesiosis and are available from some commercial reference laboratories and the CDC, although none are FDA-cleared. These methods offer similar or improved sensitivity to the thick blood film and require no specialized parasitologic expertise. NAATs may be useful in accurate diagnosis of acute infection if blood films are negative or difficult to obtain and in the differentiation of malaria parasites from Babesia or nonparasitic artifacts. Finally, NAAT may provide diagnostic confirmation in cases empirically treated without prior laboratory diagnosis by detection of remnant nucleic acid. Because residual DNA can be detected days (or even weeks to months in asplenic persons) after intact parasites have been eradicated, NAATs should not be used to monitor response to therapy. When a NAAT is positive for Plasmodium or Babesia parasites, blood films must still be examined to determine the percentage parasitemia.

It is important to stress that requests for malaria and babesiosis diagnosis should be considered "stat" and testing performed as rapidly as possible. NAAT assays may be rapid but are usually limited to the reference laboratory setting, and the total turnaround time will be too long to enable rapid institution of antimalarial therapy. In such cases, the primary use of NAATs is for confirmation of infection, assistance in species identification, and differentiation of malaria from Babesia.

B. American Trypanosomiasis (Chagas Disease) Caused by Trypanosoma cruzi ::: XV. BLOOD AND TISSUE PARASITE INFECTIONS:

American trypanosomiasis may consist of acute, latent, and chronic phases, and the optimal diagnostic method differs with each stage. The standard method for diagnosis of American trypanosomiasis during the acute phase of infection (4–8 weeks in length) is microscopy of Giemsa-stained thick and thin blood or buffy coat films, since extracellular trypanosomes will be present at this time (Table 74). As with blood films for malaria and Babesia, a minimum amount of resources (staining materials and high-quality microscopes), as well as proficient and experienced technologists, must be available to obtain maximum accuracy and efficiency. On stained preparations, the motile trypomastigote forms typically adopt a "C" shape and can be differentiated from the similar-appearing trypomastigotes of Trypanosoma brucei by the presence in T. cruzi of a large posterior kinetoplast. In comparison, the kinetoplast of T. brucei trypomastigotes is much smaller. Of course, these infections can also be likely differentiated on epidemiologic grounds. Motile organisms can also be observed in fresh wet preparations of anticoagulated blood or buffy coat, although most US laboratories are unfamiliar with this method. Unfortunately, infection is rarely diagnosed in the acute stage since only 1%–2% of infected individuals present with symptoms during this time period [289, 290].

Microscopy is less useful during the latent and chronic stages of infection when rates of parasitemia are very low. The diagnosis in these stages may be established serologically or by microscopic examination of tissue aspirates or biopsies. The nonmotile (amastigote) intracellular form of T. cruzi predominates during this phase of the infection. Culture in easily prepared Novy-MacNeal-Nicolle medium or similar media of any appropriate blood or tissue specimen during the acute and chronic stages will add to the sensitivity of laboratory diagnosis and may be available through the CDC or specialized reference laboratories. Live trypanosomes are highly infectious and specimens must be handled with care using "standard precautions" for the handling of blood and body fluids.

Serology by commercially available enzyme-linked immunoassay (ELISA) kits is of greatest use during the latent and chronic stages of disease when parasites are no longer easily detected in peripheral blood preparations by microscopy. Positive ELISA results are considered evidence of active infection and would exclude potential blood/tissue donors who test positive from acting as donors, as the infection has been shown to be transmitted by transfusion and transplantation.