Clinical Infectious Diseases

IDSA GUIDELINE

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 Microbiology[a]

J. Michael Miller,[1] Matthew J. Binnicker,[2] Sheldon Campbell,[3] Karen C. Carroll,[4] Kimberle C. Chapin,[5] Peter H. Gilligan,[6] Mark D. Gonzalez,[7] Robert C. Jerris,[7] Sue C. Kehl,[8] Robin Patel,[2] Bobbi S. Pritt,[2] Sandra S. Richter,[9] Barbara Robinson-Dunn,[10] Joseph D. Schwartzman,[11] James W. Snyder,[12] Sam Telford III,[13] Elitza S. Theel,[2] Richard B. Thomson Jr,[14] Melvin P. Weinstein,[15] and Joseph D. Yao[2]

[1]Microbiology Technical Services, LLC, Dunwoody, Georgia; [2]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota; [3]Yale University School of Medicine, New Haven, Connecticut; [4]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland; [5]Department of Pathology, Rhode Island Hospital, Providence; [6]Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill; [7]Department of Pathology, Children's Healthcare of Atlanta, Georgia; [8]Medical College of Wisconsin, Milwaukee; [9]Department of Laboratory Medicine, Cleveland Clinic, Ohio; [10]Department of Pathology and Laboratory Medicine, Beaumont Health, Royal Oak, Michigan; [11]Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire; [12]Department of Pathology and Laboratory Medicine, University of Louisville, Kentucky; [13]Department of Infectious Disease and Global Health, Tufts University, North Grafton, Massachusetts; [14]Department of Pathology and Laboratory Medicine, NorthShore University HealthSystem, Evanston, Illinois; and [15]Departments of Medicine and Pathology & Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey

Contents

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Correspondence: J. M. Miller, Microbiology Technical Services, LLC, PO Box 88212, Dunwoody, GA 30338 (jmm8@comcast.net).

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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.

Keywords. specimen management; clinical relevance; specimen collection; clinical correlation; microbiology specimens.

EXECUTIVE SUMMARY

INTRODUCTION

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

Table 1. Transport Issues (General Guide)[a]

Specimen Type	Specimen Required	Collection Device, Temperature, and Ideal Transport Time
Aerobic bacterial culture	Tissue, fluid, aspirate, biopsy, etc	Sterile container, RT, immediately
	Swab (second choice); flocked swabs are recommended	Swab transport device, RT, 2 h
Aerobic and anaerobic bacterial culture	Tissue, fluid, aspirate, biopsy, etc	Sterile anaerobic container, RT, immediately
	Swab (second choice); flocked swabs are effective	Anaerobic swab transport device, RT, 2 h
Fungus culture; AFB culture	Tissue, fluid, aspirate, biopsy, etc	Sterile container, RT, 2 h
	Swab (second choice) (for yeast and superficial mycobacterial infections only)	Swab transport device, RT, 2 h
Virus culture	Tissue, fluid, aspirate, biopsy, etc	Viral transport media, on ice, immediately
	Swab; flocked swabs are recommended	Virus swab transport device, RT, 2 h
Suspected agent of bioterrorism	Refer to CDC website for specimen collection and shipping: https://emergency.cdc.gov/labissues/index.asp	
Serology	5 mL serum	Clot tube, RT, 2 h
Antigen test	As described in the laboratory specimen collection manual	Closed container, RT, 2 h
NAAT	5 mL plasma	EDTA tube, RT, 2 h
	Other specimen, ie, viral transport medium	Closed container, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; CDC, Centers for Disease Control and Prevention; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; RT, room temperature.

[a]Contact the microbiology laboratory regarding appropriate collection and transport devices and procedures as transport media such as Cary-Blair or parasite preservative transport for stool specimens, boric acid for urines, and specialized containers for *Mycobacterium tuberculosis* are often critical for successful examination. The time from collection to transport listed will optimize results; longer times may compromise results.

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

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

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

1. Specimens of poor quality must be rejected. Microbiologists act correctly and responsibly when they call physicians to clarify and resolve problems with specimen submissions.
2. Physicians should not demand that the laboratory report "everything that grows." This can provide irrelevant information that could result in inaccurate diagnosis and inappropriate therapy.
3. "Background noise" of commensal microbiota must be avoided where possible. Many body sites have normal, commensal microbiota that can easily contaminate the inappropriately collected specimen and complicate interpretation. Therefore, specimens from sites such as lower respiratory tract (sputum), nasal sinuses, superficial wounds, fistulae, and others require care in collection.
4. The laboratory requires a specimen, not a swab of a specimen. Actual tissue, aspirates, and fluids are always specimens of choice, especially from surgery. A swab is not the specimen of choice for many specimens because swabs pick up extraneous microbes, hold extremely small volumes of the specimen (0.05 mL), and make it difficult to get bacteria or fungi away from the swab fibers and onto media, and the inoculum from the swab is often not uniform across several different agar plates. Swabs are expected from the nasopharynx and to diagnose most viral respiratory infections. Flocked swabs have become a valuable tool for specimen collection and have been shown to be more effective than Dacron, rayon, and cotton swabs in many situations. The flocked nature of the swab allows for more efficient release of contents for evaluation.
5. The laboratory must follow its procedure manual or face legal challenges. The procedures in the manuals should be supported by the literature, especially evidence-based literature. To request the laboratory to provide testing apart from the procedure manual places everyone at legal risk.
6. A specimen should be collected prior to administration of antibiotics. Once antibiotics have been started, the microbiota changes and etiologic agents are impacted, leading to potentially misleading culture results.
7. Susceptibility testing should be done only on clinically significant isolates, not on all microorganisms recovered in culture.
8. Microbiology laboratory results that are reported should be accurate, significant, and clinically relevant.
9. The laboratory should set technical policy; this is not the purview of the medical staff. Good communication and mutual respect will lead to collaborative policies.
10. Specimens must be labeled accurately and completely so that interpretation of results will be reliable. Labels such as "eye" and "wound" are not helpful to the interpretation of results without more specific site and clinical information (eg, dog bite wound right forefinger).

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

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.

I. BLOODSTREAM INFECTIONS AND INFECTIONS OF THE CARDIOVASCULAR SYSTEM

A. Bloodstream Infections and Infective Endocarditis

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

Table 2. Blood Culture Laboratory Diagnosis Organized by Etiological Agent

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Staphylococcus spp *Streptococcus* spp *Enterococcus* spp *Listeria monocytogenes* Enterobacteriaceae *Pseudomonas* spp *Acinetobacter* spp HACEK[a] bacteria *Brucella* spp Anaerobic bacteria	Adults: 2–4 blood culture sets per septic episode	20–30 mL of blood per culture set in adults injected into at least 2 blood culture bottles[b]	Inoculated culture vials should be transported to the Laboratory ASAP at RT, organisms will usually survive in inoculated culture vials even if not incubated immediately
	Infants & children: ≥2 blood culture sets	Blood volume depends on the child's weight (see Table 3)[c]	
Bartonella spp	≥2 lysis-centrifugation (Isolator) blood culture tubes[d]	10 mL of blood should be inoculated directly into each lysis-centrifugation culture tube	Lysis-centrifugation culture tubes should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation
	NAAT	5 mL of plasma	EDTA tube, RT, 2 h
	Serology for IgM/IgG antibodies	5 mL of serum	Clot tube; RT; 2 h
Legionella spp	2 or more lysis- centrifugation (Isolator) blood culture tubes[e]	10 mL of blood should be inoculated directly into each lysis-centrifugation culture tube	Lysis-centrifugation culture tubes should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation
	Legionella urine antigen test (for serotype 1)	10 mL of midstream, clean-catch urine[f]	Closed container, RT, 2 h
Coxiella burnetii	*Coxiella* IFA serology	5 mL of serum	Clot tube, RT, 2 h
	NAAT	5 mL of plasma	EDTA tube, RT, 2 h
Tropheryma whipplei	NAAT	5 mL of plasma	EDTA tube, RT, 2 h
Yeast	Adults: 2–4 blood culture sets (see above)	20–30 mL of blood per culture in adults injected into at least 2 blood culture bottles[g]	Inoculated culture vials should be transported ASAP at RT to the laboratory for early incubation. Inoculated vials for direct detection of *Candida* spp by T2 magnetic resonance assay may be used [10].
	Infants and children: ≥2 blood culture sets (see above)	As much blood as can be conveniently obtained from children; volume depends on weight of child[c]	Organisms will usually survive in inoculated culture vials even if not incubated immediately. *Malassezia* spp require lipid supplementation; lysis-centrifugation is recommended for their recovery.
Filamentous and dimorphic fungi[h]	≥2 lysis-centrifugation (Isolator) blood culture vials	10 mL of blood should be inoculated directly into each lysis-centrifugation culture vial	Lysis-centrifugation culture vials should be transported to the laboratory ASAP and processed within 8 h of blood inoculation.
Mycobacteria	3 cultures using AFB-specific blood culture bottles	5 mL of blood inoculated directly into AFB-specific blood culture bottle	Inoculated culture vials should be transported to the laboratory ASAP for early incubation.

Abbreviations: AFB, acid-fast bacilli; ASAP, as soon as possible; EDTA, ethylenediaminetetraacetic acid; IFA, indirect fluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

[a]HACEK bacteria include *Haemophilus (Aggregatibacter) aphrophilus*, *Haemophilus parainfluenzae*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*.

[b]Typically, blood specimens are split between aerobic and anaerobic blood culture bottles. There may be circumstances in which it is prudent to omit the anaerobic vial and split blood specimens between 2 aerobic vials. One example is when fungemia due to yeast is strongly suspected. Most manufacturers' bottles accept a maximum of 10 mL per bottle.

[c]Recommended volumes of blood for culture in pediatric patients are shown in (Table 3) [1].

[d]The success rate for recovery of *Bartonella* spp from blood even when optimum methods are used is extremely low.

[e]*Legionella* bacteremia occurs infrequently and rarely is the organism recovered from blood even when optimum culture techniques are employed.

[f]The optimum urine specimen is the first voided specimen of the day.

[g]Since yeast are highly aerobic, when fungemia due to yeast is suspected, it might be prudent within a series of blood cultures to inoculate at least 1 blood specimen into 2 aerobic vials rather than the customary aerobic and anaerobic vial pair. Alternatively, a broth medium designed for enhanced yield of yeast (eg, MycoF/Lytic [BD Diagnostics, Sparks, Maryland]) or lysis-centrifugation may be used.

[h]Some dimorphic fungi and yeasts (eg, *Malassezia* spp) may be visualized on peripheral blood smears in some patients using one of a variety of fungal stains. Such requests should be made in consultation with the microbiology laboratory director.

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

Table 3. Recommended Volumes of Blood for Culture in Pediatric Patients (Blood Culture Set May Use Only 1 Bottle)

Weight of Patient, kg	Total Patient Blood Volume, mL	Recommended Volume of Blood for Culture, mL		Total Volume for Culture, mL	% of Total Blood Volume
		Culture Set No. 1	Culture Set No. 2		
≤1	50–99	2	…	2	4
1.1–2	100–200	2	2	4	4
2.1–12.7	>200	4	2	6	3
12.8–36.3	>800	10	10	20	2.5
>36.3	>2200	20–30	20–30	40–60	1.8–2.7

When 10 mL of blood or less is collected, it should be inoculated into a single aerobic blood culture bottle.

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:

- Volume of blood collected, not timing, is most critical.
- Disinfect the venipuncture site with chlorhexidine or 2% iodine tincture in adults and children >2 months old (chlorhexidine NOT recommended for children <2 months old), using povidone-iodine and alcohol).
- Draw blood for culture before initiating antimicrobial therapy.
- Catheter-drawn blood cultures have a higher risk of contamination (false positives).
- Do not submit catheter tips for culture without an accompanying blood culture obtained by venipuncture.
- Never refrigerate blood prior to incubation.
- Use a 2- to 3-bottle blood culture set for adults, at least 1 aerobic and 1 anaerobic; use 1–2 aerobic bottles for children and consider aerobic and anaerobic when clinically relevant.
- *Streptococcus pneumoniae* and other gram-positive organisms and facultatively anaerobic organisms may grow best in the anaerobic bottle (faster time to detection).

B. Infections Associated With Vascular Catheters

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:

- Time to positivity (not performed routinely in most laboratories): Standard blood cultures (BCs) obtained at the same time, one from the catheter or port and one from peripheral venipuncture, processed in a continuous-monitoring BC system. If both BCs grow the same organism and the BC drawn from the device becomes positive >2 hours before the BC drawn by venipuncture, there is a high probability of catheter-associated BSI [14].

- Quantitative BCs (not performed routinely in most laboratories): one from catheter or port and one from peripheral venipuncture obtained at the same time using lysis-centrifugation (Isolator) or pour plate method. If both BCs grow the same organism and the BC drawn from the device has 5-fold more organisms than the BC drawn by venipuncture, there is a high probability of catheter-associated BSI [15, 16].
- Catheter tip or segment cultures: The semi-quantitative method of Maki et al [12] is used most commonly; interpretation requires an accompanying peripheral BC. However, meticulous technique is needed to reduce contamination and to obtain the correct length (5 cm) of the distal catheter tip. This method only detects organisms colonizing the outside of the catheter, which is rolled onto an agar plate, after which the number of colonies is counted; organisms that may be intraluminal are missed. Modifications of the Maki method have been described as have methods that utilize vortexing of the catheter tip or an endoluminal brush (not performed routinely in most laboratories). Biofilm formation on catheter tips prevents antimicrobial therapy from clearing agents within the biofilm, thus requiring removal of the catheter to eliminate the organisms.

C. Infected (Mycotic) Aneurysms and Vascular Grafts

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

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.

II. CENTRAL NERVOUS SYSTEM INFECTIONS

Clinical microbiology tests of value in establishing an etiologic diagnosis of infections within the central nervous system (CNS) are outlined below. In this section, infections are categorized as follows: meningitis, encephalitis, focal infections of brain parenchyma, CNS shunt infections, subdural empyema, epidural abscess, and suppurative intracranial thrombophlebitis.

Organisms usually enter the CNS by crossing a mucosal barrier into the bloodstream followed by penetration of the blood–brain barrier. Other routes of infection include direct extension from a contiguous structure, movement along nerves, or introduction by foreign devices.

Usually 3 or 4 tubes of cerebrospinal fluid (CSF) are collected by lumbar puncture for diagnostic studies. The first tube has the highest potential for contamination with skin flora and should not be sent to the microbiology laboratory for direct smears, culture, or molecular studies. A minimum of 0.5–1 mL of CSF should be sent immediately after collection to the microbiology laboratory in a sterile container for bacterial testing. Larger volumes (5–10 mL) increase the sensitivity of culture and are required for optimal recovery of mycobacteria and fungi. When the specimen volume is less than required for multiple test requests, prioritization of testing must be provided to the laboratory. Whenever possible, specimens for culture should be obtained prior to initiation of antimicrobial therapy.

Table 4. Laboratory Methods for Diagnosis of Infected Aneurysms and Vascular Grafts

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria	Gram stain Aerobic bacterial culture[a] Blood cultures (see Section I-A)	Lesion biopsy or resected graft material[b]	Sterile container, RT, immediately
Fungi	Calcofluor-KOH stain[c] Fungal culture Blood cultures (see Section I-A)	Lesion biopsy or resected graft material[b]	Sterile container, RT, 2 h

Abbreviations: KOH, potassium hydroxide; RT, room temperature.

[a]If aerobic bacteria are suspected. If anaerobes are suspected, then the culture should consist of an aerobic and anaerobic bacterial culture.

[b]Tissue specimens or a portion of the graft material are always superior to swab specimens of infected sites, even when collected using sterile technique during surgery.

[c]Calcofluor stain is a fluorescent stain and requires special microscopy equipment and may not be available at all facilities.

Table 5. Laboratory Diagnosis of Pericarditis and Myocarditis

Etiologic Agents[a]	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria	Gram stain Aerobic bacterial culture[b] Blood cultures (see I-a above)	Pericardial fluid or pericardium biopsy	Sterile container or blood culture vial (pericardial fluid only), RT, immediately
Fungi	Calcofluor-KOH stain Fungal culture Blood cultures (see Section I-A)	Pericardial fluid or pericardium biopsy	Sterile container, RT, 2 h
Mycobacteria	Acid-fast smear AFB culture Blood cultures (see Section I-A)	Pericardial fluid or pericardium biopsy[c]	Sterile container, RT, 2 h
Viruses			
Coxsackie B virus Coxsackie A virus Echovirus Polio virus Adenovirus HIV Mumps virus Cytomegalovirus Other viruses	Virus-specific serology	Acute and convalescent sera	Clot tube, RT, 2 h
	Virus-specific NAAT (may be first choice if test is available)	Pericardial fluid or pericardium biopsy	Closed container, RT, 2 h
	Virus culture (culture not productive for all virus types)	Pericardial fluid or pericardium biopsy	Virus transport device, on ice, immediately
	Histopathologic examination	Pericardial fluid or pericardium biopsy	Place in formalin and transport to histopathology laboratory for processing.
Parasites[d]			
Trypanosoma cruzi *Trichinella spiralis* *Toxoplasma gondii*	Parasite-specific serology	Acute and convalescent sera	Clot tube, RT, 2 h
	Blood smear[e]	5 mL of peripheral blood	EDTA tube, RT
	Histopathologic examination *Toxoplasma* NAAT	Endomyocardial biopsy or surgical specimen	Consultation with the laboratory is recommended. For histopathology, place in formalin and transport to histopathology laboratory for processing.

Abbreviations: AFB, acid-fast bacilli; EDTA, ethylenediaminetetraacetic acid; HIV, human immunodeficiency virus; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

[a]Other infectious causes of pericarditis and myocarditis include rickettsiae (*Rickettsia rickettsii*, *Coxiella burnetii*), chlamydiae, *Borrelia burgdorferi*, *Treponema pallidum*, *Nocardia* spp, *Tropheryma whipplei*, *Legionella pneumophila*, *Actinomyces* spp, *Entamoeba histolytica*, *Ehrlichia* spp, *Toxocara canis*, *Schistosoma*, and *Mycoplasma* spp.

[b]If aerobic bacteria are suspected. If anaerobes are suspected, then the culture should consist of both a routine aerobic and anaerobic culture.

[c]Pericardial tissue is superior to pericardial fluid for the culture recovery of *Mycobacterium* spp.

[d]If parasites other than *T. cruzi*, *T. gondii*, or *T. spiralis* are suspected, consult the Centers for Disease Control and Prevention Parasitic Consultation Service (http://dpd.cdc.gov/dpdx/HTML/Contactus.htm).

[e]Blood smears may be useful in detection of infection caused by *Trypanosoma* spp.

CSF Gram stains should be prepared after cytocentrifugation and positive results called to the patient care area immediately. Identification and susceptibility testing of bacteria recovered from cultures is routinely performed unless contamination during collection or processing is suspected.

Most clinical microbiology laboratories do not perform all of the testing listed in the tables. This is especially true of serologic and many molecular diagnostic tests. The availability of US Food and Drug Administration (FDA)–cleared NAATs for many agents is limited, requiring laboratory-developed tests to be used, with variable sensitivities and specificities. Although an FDA-cleared multiplex PCR targeting 14 organisms is available for diagnosing meningitis and encephalitis, it should not be considered a replacement for culture since clinical experience with the assay is limited and specificity issues have been reported [17, 18]. The expense and data interpretation challenges associated with next-generation sequencing have prevented widespread use of this technology for the time being [19]. Serologic diagnosis is based on CSF to serum antibody index, 4-fold rise in acute to convalescent immunoglobulin G (IgG) titer, or a single positive immunoglobulin M (IgM). Detection of antibody in CSF may indicate CNS infection, blood contamination, or transfer of antibodies across the blood–brain barrier. Submission of acute (3–10 days after onset of symptoms) and convalescent (2–3 weeks after acute) serum samples is recommended. Serum should be separated from red cells as soon as possible.

Key points for the laboratory diagnosis of CNS infections:

- Whenever possible, collect specimens prior to initiating antimicrobial therapy.
- Two to 4 BCs should also be obtained if bacterial meningitis is suspected.
- Inform the microbiology laboratory if unusual organisms are possible (eg, *Nocardia*, fungi, mycobacteria), for which special procedures are necessary. [20].
- Do not refrigerate CSF.
- CSF tubes #2 or #3, not #1, should be submitted for bacterial culture and molecular testing.
- Attempt to collect as much sample as possible for multiple studies (minimum recommended is 1 mL); prioritize multiple test requests on small-volume samples.

A. Meningitis

The most common etiologic agents of acute meningitis are viruses (echoviruses and parechoviruses) and bacteria (*Streptococcus*

Table 6. Laboratory Diagnosis of Meningitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Streptococcus pneumoniae *Neisseria meningitidis* *Listeria monocytogenes* *Streptococcus agalactiae* *Haemophilus influenzae* *Escherichia coli* Other Enterobacteriaceae *Elizabethkingia meningoseptica* *Citrobacter diversus*	Gram stain[a] Aerobic bacterial culture Blood cultures	CSF Blood, 2–4 sets	Sterile container, RT, immediately Blood culture bottles, RT, 2 h
Mycobacterium tuberculosis	AFB smear AFB culture	CSF (≥5 mL)	Sterile container, RT, 2 h
	Mycobacterium tuberculosis NAAT[b]	CSF	Sterile container, RT, 2 h
Spirochetal			
Treponema pallidum (syphilis)	VDRL, FTA-ABS	CSF	Sterile container, RT, 2 h
	Traditional: RPR screening test with positive RPR confirmed by TPPA test or other treponemal confirmatory test Reverse sequence: EIA or chemiluminescent immunoassay treponemal screening test with positive confirmed by RPR (negative RPR reflexed to TPPA)	Serum	Clot tube, RT, 2 h
Borrelia burgdorferi (Lyme disease)	*B. burgdorferi* antibodies, IgM and IgG with Western blot assay confirmation[c]	Serum	Clot tube, RT, 2 h
		CSF	Closed container, RT, 2 h
	B. burgdorferi NAAT (low sensitivity)	CSF	Sterile container, RT, 2 h
Leptospira spp	*Leptospira* NAAT[d]	Blood	EDTA or sodium citrate tube, RT, 2 h
		CSF, urine	Sterile container, RT, 2 h
	Leptospira culture (special media required; rarely available in routine laboratories)	First week of illness: CSF, 10 mL blood	Sterile container, heparin or citrate tube, RT, immediately
		After first week of illness: 10 mL urine (neutralized)	Sterile container, RT, immediately
	Leptospira antibody, microscopic agglutination test	Serum	Clot tube, RT, 2 h
Fungal			
Cryptococcus neoformans	*Cryptococcus* antigen test	CSF	Closed container, RT, 2 h
Cryptococcus gattii	Gram stain Aerobic bacterial culture (faster growth on blood agar medium) Fungal culture	CSF	Sterile container, RT, 2 h
Coccidioides spp	*Coccidioides* antibody, complement fixation, and immunodiffusion[e] Calcofluor stain and Fungal culture	CSF Serum CSF	Closed container, RT, 2 h Clot tube, RT, 2 h Sterile container, RT, 2 h
Parasitic			
Acanthamoeba spp *Naegleria fowleri*	See Table 7		
Viral			
Enteroviruses (nonpolio)	Enterovirus NAAT	CSF	Sterile container, RT, 2 h
Parechoviruses	Parechovirus NAAT	CSF	Sterile container, RT, 2 h
Herpes simplex virus	HSV-1 and HSV-2 NAAT	CSF	Sterile container, RT, 2 h
Varicella zoster virus	VZV NAAT	CSF	Sterile container, RT, 2 h
LCM virus	LCM antibodies, IgM and IgG, IFA	CSF	Closed container, RT, 2 h
		Serum	Clot tube, RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
		CSF	Closed container, RT, 2 h
	Mumps culture and NAAT	CSF	Sterile container, on ice, immediately
		Buccal or oral swab[f]	Viral transport device, on ice, immediately
HIV	[g]		

Abbreviations: AFB, acid-fast bacilli; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody–absorbed; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IFA, indirect fluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; LCM, lymphocytic choriomeningitis virus; NAAT, nucleic acid amplification test; RPR, rapid plasma reagin; RT, room temperature; TPPA, *Treponema pallidum* particle agglutination assay; VDRL, Venereal Disease Research Laboratory; VZV, varicella zoster virus.

[a]Gram stains may be performed on uncentrifuged specimens when the CSF is visibly turbid.

[b]A negative result does not rule out *Mycobacterium tuberculosis*.

[c]Include a CSF index: simultaneous CSF:serum ratio of *Borrelia burgdorferi* antibodies with normalized protein amounts.

[d]The Centers for Disease Control and Prevention accepts specimens referred by state or local public health laboratories (https://www.cdc.gov/laboratory/specimen-submission/index.html).

[e]Complement fixation on CSF is optimal test; serum complement fixation antibody may reflect a remote rather than an active infection.

[f]Specimen collection instructions available at https://www.cdc.gov/mumps/lab/specimen-collect.html.

[g]The diagnosis of acute meningitis due to HIV, a condition that often arises during the early stages of the HIV retroviral syndrome, is best established based on compatible CSF findings (ie, a mild CSF lymphocytosis with a mildly elevated CSF protein level and normal glucose) combined with definitive evidence of recent HIV infection (see Section XIV, HIV diagnosis).

Table 7. Laboratory Diagnosis of Encephalitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Viral			
Herpes simplex virus	HSV-1 and -2 NAAT	CSF	Sterile container, RT, 2 h
Enteroviruses (nonpolio)	Enterovirus NAAT	CSF	Sterile container, RT, 2 h
Parechoviruses	Parechovirus NAAT	CSF	Sterile container, RT, 2 h
West Nile virus	WNV IgM antibody[a]	CSF and/or serum	Closed container or clot tube, RT, 2 h
	WNV NAAT[b]	CSF and/or serum	Sterile container, RT, 2 h
Other arboviruses[c]	Virus specific antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
Varicella zoster virus[d]	VZV NAAT	CSF or plasma	Sterile container or EDTA tube, RT, 2 h
	VZV antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
Epstein-Barr virus	EBV NAAT[e]	CSF or plasma	Sterile container or EDTA tube, RT, 2 h
	EBV antibodies, VCA IgG and IgM, EBNA	CSF and/or serum	Closed container or clot tube, RT, 2 h
Cytomegalovirus[f]	CMV NAAT[g]	CSF or plasma	Sterile container or EDTA tube, RT, 2 h
	CMV antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
Human herpesvirus 6	HHV-6 NAAT	CSF	Sterile container, RT, 2 h
JC virus	JC virus NAAT	CSF	Sterile container, RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
		CSF	Closed container, RT, 2 h
	Mumps culture and NAAT	CSF	Sterile container, on ice, immediately
		Buccal or oral swab	Viral transport device, on ice, immediately
Measles (rubeola) virus	Measles antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
	Measles culture and NAAT	CSF, urine	Sterile container, RT, 2 h
		Throat swab	Viral transport device, on ice, immediately
Influenza virus	Influenza DFA and culture or NAAT	Nasopharyngeal wash or other respiratory specimen	Viral transport device, on ice, immediately
Adenovirus	Adenovirus DFA and culture or NAAT	Nasopharyngeal wash or other respiratory specimen	Viral transport device, on ice, immediately
	Adenovirus NAAT	CSF or plasma	Sterile container or EDTA, RT, 2 h
Rabies virus[h]	Rabies antigen, DFA	Nuchal skin biopsy	Closed container, RT, immediately
	Rabies NAAT	Saliva	Sterile container, RT, immediately
	Rabies antibody	CSF and serum	Closed container, clot tube, RT, 2 h
Lymphocytic choriomeningitis virus	LCM antibodies, IgM and IgG, IFA	CSF and/or serum	Closed container or clot tube, RT, 2 h
Zika virus (see Table 71)			
Bacterial			
Mycobacterium tuberculosis	See Table 6, Meningitis		
Bartonella spp	*Bartonella* spp NAAT	CSF or plasma	Sterile container or EDTA, RT, 2 h
	Bartonella spp antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
Mycoplasma pneumoniae	*M. pneumoniae* NAAT	CSF or respiratory	Sterile container, RT, 2 h
	M. pneumoniae antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
Tropheryma whipplei (Whipple disease)	*T. whipplei* NAAT	CSF	Sterile container, RT, 2 h
Listeria monocytogenes	Gram stain Aerobic bacterial culture	CSF, blood	Sterile container, aerobic blood culture bottle, RT, 2 h
	Listeria antibody, complement fixation	CSF and/or serum	Closed container or clot tube, RT, 2 h
Coxiella burnetii (Q fever)	*C. burnetii* antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
	C. burnetii NAAT	Whole blood	EDTA tube, RT, 2 h
		Tissue	Sterile container, RT, 2 h
Rickettsia rickettsii (RMSF), *Rickettsia typhi*	*Rickettsia* spp antibodies, IgG and IgM, IFA	CSF and/or serum	Closed container or clot tube, RT, 2 h
	R. rickettsii DFA or IHC and NAAT	Skin biopsy from rash	Sterile container, RT, 2 h
	R. rickettsii NAAT	Whole blood	EDTA tube, RT, 2 h
Ehrlichia chaffeensis, *Anaplasma phagocytophilum*	*E. chaffeensis* and *A. phagocytophilum* antibodies, IgM and IgG	CSF and/or serum	Closed container or clot tube, RT, 2 h
	E. chaffeensis and *A. phagocytophilum* NAAT	Whole blood	EDTA tube, RT, 2 h

Table 7. Continued

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Other: *B. burgdorferi*, *T. pallidum*, *Leptospira* spp	See Table 6, Meningitis		
Fungal			
Cryptococcus neoformans	Cryptococcus antigen test	CSF, serum	Closed container, clot tube, RT, 2 h
Cryptococcus gattii	Gram stain Aerobic bacterial culture Fungal culture	CSF	Sterile container, RT, 2 h
Coccidioides spp	*Coccidioides* antibody, immunodiffusion, and complement fixation[e]	CSF and/or serum	Closed container or clot tube, RT, 2 h
	Calcofluor stain Fungal culture	CSF, other sites	Sterile container, RT, 2 h
	Histologic examination	Tissue or formalin-fixed tissue	Sterile container, RT, 2 h or formalin, indefinite
Parasitic			
Acanthamoeba spp *Naegleria fowleri*	Microscopic wet mount Giemsa stain	CSF	Closed container, RT, 2 h
	Histology (trichrome stain)	CSF, brain tissue	Closed container, RT, 2 h
	Culture	CSF, brain tissue	Sterile container, RT, 2 h
	Acanthamoeba antibody IFA[i]	Serum	Clot tube, RT, 2 h
	Acanthamoeba IIF staining[i]	Brain tissue	Closed container, RT, 2 h
	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h
Balamuthia mandrillaris	*Balamuthia* antibody, IFA[i]	Serum	Clot tube, RT, 2 h
	Balamuthia IIF staining[i]	Brain tissue	Closed container, RT, 2 h
Baylisascaris procyonis[j]	*B. procyonis* antibodies	CSF and/or serum	Closed container or clot tube, RT, 2 h
Trypanosoma brucei spp	Giemsa stain	CSF, brain tissue	Closed container, RT, 2 h
		Blood	EDTA tube, RT, 2 h
Toxoplasma gondii	*Toxoplasma* NAAT	CSF, serum, plasma	Sterile container, clot tube, EDTA tube, RT, 2 h
	Toxoplasma antibodies, IgM and IgG[k]	CSF and/or serum	Closed container or clot tube, RT, 2 h
	Giemsa stain, histology	CSF, brain tissue	Closed container, RT, 2 h
Prion			
Creutzfeldt-Jakob disease[l]	14-3-3 protein	CSF	Closed container, RT, 2 h
	Neuron-specific enolase	CSF	Closed container, RT, 2 h
	Routine histology, immune stain for prion protein	Formalin-fixed brain tissue	Contact surgical pathologist prior to collection of tissue[m]
	Western blot for prion protein	Frozen brain tissue	Contact surgical pathologist prior to collection of tissue[m]
	PrP gene sequencing	Blood, other tissues	EDTA tube, sterile container, RT, 2 h

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; EDTA, ethylenediaminetetraacetic acid; HHV-6, human herpesvirus type 6; HSV, herpes simplex virus; IFA, indirect fluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; IHC, immunohistochemistry; IIF, indirect immunofluorescent antibody; LCM, lymphocytic choriomeningitis virus; NAAT, nucleic acid amplification testing; RMSF, Rocky Mountain spotted fever; RT, room temperature; VCA, viral capsid antigen; WNV, West Nile virus.

[a]WNV IgM antibody may persist for >6 months. False positives may occur with recent immunization (Japanese encephalitis, yellow fever) or other flavivirus infection (dengue, St Louis encephalitis, Zika) [30].

[b]Sensitivity of WNV NAAT in immunocompetent host is <60% [30]. Testing for IgM in CSF is preferred, but may be falsely negative during first week of symptoms. Persistent viremia in immunocompromised hosts lacking serologic response may improve WNV NAAT sensitivity.

[c]Eastern equine, Western equine, Lacrosse, St Louis, and California encephalitis viruses.

[d]Detection of VZV DNA in CSF (~60% of cases), CSF IgM, or intrathecal antibody synthesis distinguishes meningoencephalitis from postinfectious, immune-mediated process [27].

[e]Quantitative EBV NAAT may help distinguish true-positive from latent virus [27].

[f]Congenital disease in newborns and reactivation in immunocompromised hosts. False-positive CSF CMV NAAT results have been reported in immunocompetent patients with bacterial meningitis [27].

[g]In human immunodeficiency virus–infected patients, detection of CMV DNA in CSF has 82%–100% sensitivity and 86%–100% specificity for diagnosing central nervous system CMV infection [27].

[h]Contact state public health department to arrange testing; questions regarding sampling techniques and shipping may be directed to the Rabies section at the Centers for Disease Control and Prevention (CDC), telephone: (404) 639–1050.

[i]Available at the California State Department of Health Services and the CDC [31].

[j]Consider if eosinophilia or exposure to raccoon feces [32]. Testing available at the Department of Veterinary Pathobiology, Purdue University (West Lafayette, Indiana), telephone: (765) 494-7558.

[k]Refer positive IgM to *Toxoplasma* Serology Laboratory in Palo Alto, California, for confirmatory testing (http://www.pamf.org/serology/). The absence of serum IgM or IgG does not exclude *Toxoplasma* infection (22% of AIDS patients with *Toxoplasma* encephalitis lack IgG; IgM is rarely detected) [33].

[l]Testing available at the National Prion Disease Pathology Surveillance Center (http://www.cjdsurveillance.com) [34]. The 14-3-3 protein has limited specificity for prion disease.

[m]Compliance with appropriate infection control protocols is essential.

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

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

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

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

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

Table 8. Laboratory Diagnosis of Focal Parenchymal Brain Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Aerobes: *Streptococcus*, *Staphylococcus*, Enterobacteriaceae, *Pseudomonas*, *Haemophilus*, *Listeria* spp Anaerobes: *Bacteroides*, *Fusobacterium*, *Prevotella*, *Actinomyces*, *Clostridium*, *Propionibacterium* spp	Gram stain Aerobic and anaerobic bacterial culture	Aspirate of abscess contents, tissue	Sterile anaerobic container, RT, immediately
Nocardia spp	Gram stain, modified acid-fast stain Aerobic bacterial culture (hold 7 d; add buffered charcoal yeast extract agar)	Aspirate of abscess contents, tissue	Sterile container, RT, immediately
	Histology (GMS, Gram stain)	Tissue	Closed container, RT, 2 h
Mycobacterium tuberculosis	AFB smear AFB culture	Aspirate of abscess contents (no swabs), tissue	Sterile container, RT, 2 h
	Histology (AFB stain)	Tissue	Closed container, RT, 2 h
	M. tuberculosis NAAT[a]	Aspirate, tissue	Sterile container, RT, 2 h
Fungal			
Candida spp *Cryptococcus* spp *Aspergillus* spp Zygomycetes (*Rhizopus*, *Mucor* spp) *Scedosporium apiospermum* *Trichosporon* spp *Trichoderma* spp Dematiaceous molds (*Cladophialophora bantiana*, *Bipolaris* spp, *Exophiala* spp Endemic dimorphic fungi	Calcofluor stain Fungal culture	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Histology (GMS stain) Mucicarmine stain for *Cryptococcus*	Tissue	Closed container, RT, 2 h
Parasitic			
Toxoplasma gondii	*Toxoplasma* NAAT	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Toxoplasma antibodies, IgM and IgG[b]	Serum	Clot tube, RT, 2 h
	Giemsa stain Histology	Aspirate of abscess contents, tissue	Closed container, RT, 2 h Formalin, indefinite
Taenia solium (neurocysticercosis)	*T. solium* antibodies, IgG, ELISA, confirmatory Western blot[c]	Serum	Clot tube, RT, 2 h
	Histology[d]	Brain tissue	Closed container, RT, 2 h Formalin, indefinite
Acanthamoeba spp	Microscopic wet mount Giemsa stain	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	Histology (trichrome stain)	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	Culture	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Acanthamoeba antibody, IFA[e]	Serum	Clot tube, RT, 2 h
	Acanthamoeba IIF staining[e]	Brain tissue	Closed container, RT, 2 h
	Giemsa	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
Balamuthia mandrillaris	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h Formalin, indefinite
	Balamuthia antibody, IFA[e]	Serum	Clot tube, RT, 2 h
	Balamuthia IIF staining[e]	Brain tissue	Closed container, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; ELISA, enzyme-linked immunosorbent assay; GMS, Gomori methenamine silver; IFA, indirect fluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; IIF, indirect immunofluorescent antibody; NAAT, nucleic acid amplification test; RT, room temperature.

[a]A negative result does not rule out *Mycobacterium tuberculosis*.

[b]Refer positive IgM to *Toxoplasma* Serology Laboratory in Palo Alto, California, for confirmatory testing (http://www.pamf.org/serology/). The absence of IgM or IgG does not exclude *Toxoplasma* infection [33].

[c]Only 50% sensitivity if patient has solitary parenchymal lesion [34]; potential for false-positive ELISA results due to cross-reactivity with *Echinococcus*.

[d]Diagnosis usually on basis of clinical presentation, neuroimaging, and serology. Only occasionally are invasive procedures (brain biopsy) required.

[e]Available at the California State Department of Health Services and the Centers for Disease Control and Prevention [31].

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

Table 9. Laboratory Diagnosis of Central Nervous System Shunt Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial (1 organism or mixed)			
Aerobes: *Staphylococcus*, *Streptococcus*, Enterobacteriaceae, *Pseudomonas*, *Acinetobacter*, *Corynebacterium* spp Anaerobes: *Cutibacterium* (*Propionibacterium*) *acnes*	Gram stain Aerobic and anaerobic bacterial culture (hold 14 d for *C. acnes*)	CSF	Sterile, anaerobic container, RT, immediately
Mycobacterium spp (rare)	AFB smear AFB culture	CSF (≥5 mL)	Sterile container, RT, 2 h
Fungal			
Candida spp, other fungi	Calcofluor stain Fungal culture	CSF	Sterile container, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; CSF, cerebrospinal fluid; RT, room temperature.

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.

III. OCULAR INFECTIONS

The spectrum of ocular infections can range from superficial, which may be treated symptomatically or with empiric topical antimicrobial therapy, to those sight-threatening infections that require aggressive surgical intervention and either topical and/or parenteral antimicrobial therapy. Infections may occur in the anatomical structures surrounding the eye (conjunctivitis, blepharitis, canaliculitis, dacryocystitis, orbital and periorbital cellulitis), on the surface of the eye (keratitis), or within the globe of the eye (endophthalmitis and uveitis/retinitis). Recommendations for the laboratory diagnosis of ocular infections are often based on studies where only small numbers of clinical specimens were examined so the evidence base for many recommendations is limited. Studies comparing multiple diagnostic approaches to determine the optimal means for detection of the infectious etiology of keratitis and endophthalmitis are further hampered by small specimen size. Finally, frequent pretreatment with topical antibacterial agents further complicates laboratory diagnosis of both bacterial conjunctivitis and keratitis [36].

Key points for the laboratory diagnosis of ocular infections:

- Specimens should be labeled with the specific anatomic source, ie, conjunctiva or cornea, but not just "eye."

Table 10. Laboratory Diagnosis of Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Aerobes: *Streptococcus*, *Enterococcus*, *Staphylococcus*, Enterobacteriaceae, *Haemophilus*, *Pseudomonas* spp Anaerobes: *Peptostreptococcus*, *Veillonella*, *Bacteroides*, *Fusobacterium*, *Prevotella* spp, *Cutibacterium* (*Propionibacterium*) *acnes*	Gram stain Aerobic and anaerobic bacterial culture	Aspirate of purulent material (never use swabs)	Sterile, anaerobic container, RT, immediately
Nocardia spp	Gram stain, modified acid-fast stain Aerobic bacterial culture (hold 7 d; add BCYE agar)	Aspirate of purulent material	Sterile container, RT, immediately
Mycobacterium spp	AFB smear AFB culture *M. tuberculosis* NAAT[a] (rarely available)	Aspirate of purulent material	Sterile container, RT, 2 h
Fungal			
Candida spp, other fungi	Calcofluor stain Fungal culture	Aspirate of purulent material	Sterile container, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; BCYE, buffered charcoal yeast extract; NAAT, nucleic acid amplification test; RT, room temperature.

[a]Negative NAAT for tuberculosis does not rule out *Mycobacterium tuberculosis*.

- The Gram stain is useful in the diagnosis of conjunctivitis, so 2 swabs per site may be appropriate; a paired specimen from the uninfected eye can be used as a "control" to assist in culture or Gram stain interpretation.
- Swab specimens are routinely used but provide a minimum amount of material. Consult the laboratory regarding suspicious agents. Corneal scrapings are preferred for keratitis diagnosis.
- Organisms that are part of the indigenous microflora are usually not involved in conjunctivitis but may be involved in postsurgical keratitis and endophthalmitis.

A. Specimen Collection, Processing, and Transport

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

Table 11. Laboratory Diagnosis of Periocular Structure Infections/Conjunctivitis, Orbital and Periorbital Cellulitis, and Lacrimal and Eyelid Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Haemophilus influenzae *Streptococcus pneumoniae* *Staphylococcus aureus* *Moraxella catarrhalis* and other spp *Streptococcus pyogenes* *Escherichia coli* Other Enterobacteriaceae *Neisseria gonorrhoeae*	Gram stain Aerobic bacterial culture	Conjunctival swab	Swab transport device, RT, 2 h
Actinomyces spp Other anaerobic bacteria (rare cause of canaliculitis)	Anaerobic bacterial culture	Conjunctival scraping or biopsy	Sterile anaerobic container, RT, immediately
Chlamydia trachomatis	Direct fluorescent antibody stain NAAT[a,b]	Conjunctival swab	Virus swab transport device, RT, 2 h
Viruses			
HSV	HSV NAAT HSV culture	Conjunctival swab	Virus swab transport device, RT, 2 h
VZV	VZV NAAT	Conjunctival swab	Virus swab transport device, RT, 2 h
Adenovirus	Adenovirus NAAT	Conjunctival swab	Virus swab transport device, RT, 2 h
Herpes B virus	[c]		

Abbreviations: HSV, herpes simplex virus; NAAT, nucleic acid amplification test; RT, room temperature; VZV, varicella zoster virus.

[a]NAATs for detection of *C. trachomatis* have not yet been approved in the United States for use with conjunctival swab specimens. Individual laboratories, however, may have validated NAATs for examination of specimens obtained from patients with conjunctivitis, and studies suggest that NAATs are more sensitive than cultures.

[b]Use of NAAT for detection of *C. trachomatis* is considered an "off-label" use of this test. Laboratories that offer such testing must conduct in-house validation of these assays before offering NAAT as a diagnostic test.

[c]Culturing of specimens thought to harbor herpes B virus (primate origin) requires use of biosafety level 4 precautions in the laboratory, and testing is almost always referred to a specialized reference laboratory. Consult the laboratory when herpes B virus is suspected.

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

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

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

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

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

Table 12. Laboratory Diagnosis of Periocular Structure Infections/Keratitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial[a]			
Coagulase-negative staphylococci *Pseudomonas aeruginosa* *Cutibacterium (Propionibacterium) acnes* *Streptococcus pneumoniae* *Staphylococcus aureus* *Serratia marcescens* *Acinetobacter* spp *Escherichia coli* *Enterobacter cloacae* *Haemophilus influenzae* *Klebsiella pneumoniae* Other gram-negative bacteria *Corynebacterium* spp *Neisseria gonorrhoeae* *Nocardia* spp[c]	Gram stain[b] Aerobic bacterial culture (with bedside inoculation of plates)[b] Add BCYE agar for *Nocardia*	Corneal scrapings, sterile impression membrane, corneal biopsy	Inoculated plates and prepared slide transported directly to the laboratory[b], RT, immediately
	Anaerobic culture (for *C. acnes*)	Corneal scrapings, sterile impression membrane, corneal biopsy	Place second sample into anaerobic broth (at bedside) provided by laboratory
Mycobacterium spp[d]	Acid-fast smear AFB culture	Corneal scrapings, sterile impression membrane, corneal biopsy	Sterile container, RT, 2 h
Fungal			
Aspergillus spp *Fusarium* spp Dematiaceous fungi	Calcofluor-KOH stain[e] Fungal culture (with bedside inoculation of plates)[e]	Corneal scrapings, sterile impression membrane, corneal biopsy	Inoculated plates and prepared slide transported directly to the laboratory[e], RT, immediately
Viral			
HSV	HSV NAAT (for initial diagnosis) HSV culture	Corneal swab, corneal biopsy	Virus swab transport device, RT, 2 h
VZV	VZV NAAT	Corneal swab, Corneal biopsy	Virus swab transport device, RT, 2 h
Adenovirus	Adenovirus NAAT	Corneal swab, Corneal biopsy	Viral swab transport device, RT, 2 h
Parasites			
Acanthamoeba spp	Giemsa stain Calcofluor-KOH stain	Corneal scrapings, sterile impression membrane	Sterile container, RT, immediately. Transport in Page saline.
	Acanthamoeba NAAT or culture (with bedside inoculation of culture plate)[f]	Corneal swab, Corneal biopsy	Inoculated plate transported directly to the laboratory[f], RT, immediately. Swab/tissue for NAAT in viral transport device or saline, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; BCYE, buffered charcoal yeast extract; HSV, herpes simplex virus; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature; VZV, varicella zoster virus.

[a]The relative likelihood of a specific etiology depends on the underlying reason for the development of keratitis.

[b]Culture plates, including a sheep blood agar plate and a chocolate agar plate, should be inoculated directly with material collected on the Kimura spatula directly at the patient's bedside at the time corneal scrapings are obtained, usually applied to the agar surface as a number of small "C"-shaped inocula. If sufficient sample is available, a smear on a glass slide may also be prepared at the patient's bedside after the plates are inoculated. The inoculated plates and slide (if prepared) are then transported directly to the microbiology laboratory.

[c]The laboratory should be notified when *Nocardia* spp is suspected so that culture plates may be incubated for longer periods than normal, thus enhancing the chance of recovering this slow-growing organism. Additional media, such as BCYE, can enhance recovery of *Nocardia*.

[d]Acid-fast smears and mycobacterial cultures should be performed in all postoperative infections of the cornea. *Mycobacterium chelonae* is a common finding in such cases.

[e]At least one culture plate or slant containing a nonselective fungal growth medium should be inoculated directly at the patient's bedside at the time corneal scrapings are obtained. If sufficient sample is available, a smear on a glass slide may also be prepared at the patient's bedside. This should be attempted only after plates/slants have been inoculated. The inoculated plates/slants and slide (if prepared) are then transported directly to the microbiology laboratory. The smear is stained with Calcofluor-KOH in the laboratory and examined for fungal elements.

[f]A corneal swab specimen is used to inoculate an agar plate containing nonnutritive medium at the patient's bedside and then transported immediately to the laboratory. In the laboratory, the plate is overlaid with a lawn of viable *Escherichia coli* or some other member of the Enterobacteriaceae (ie, co-cultivation) prior to incubation. Alternatively, plates seeded with the bacteria are inoculated with a bit of corneal scraping material or a drop of a suspension of the scraped sample in sterile saline.

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

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

Table 13. Laboratory Diagnosis of Endophthalmitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial[a]			
Coagulase-negative staphylococci *Staphylococcus aureus* *Streptococcus agalactiae* Viridans streptococci *Bacillus cereus* and related spp *Pseudomonas aeruginosa* *Acinetobacter* spp *Escherichia coli* *Enterobacter cloacae* *Haemophilus influenzae* *Serratia marcescens* *Klebsiella pneumoniae* *Neisseria meningitidis* *Enterococcus* spp *Listeria monocytogenes* *Cutibacterium (Propionibacterium) acnes* *Corynebacterium* spp *Nocardia* spp[c]	Gram stain[b] Aerobic bacterial culture (with bedside inoculation of plates)[b] Add BCYE agar for *Nocardia*	Aqueous aspirate Vitreous aspirate, washing or biopsy	Inoculated plates and prepared slide transported directly to the laboratory[b], RT, immediately Washing sent to laboratory, RT, 2 h
	Anaerobic culture for *C. acnes*	Place second sample into anaerobic broth (at bedside) provided by laboratory	Sterile anaerobic container, RT, immediately
Mycobacteria			
Mycobacterium spp[d]	Acid-fast smear[e] AFB culture (with bedside inoculation of slants)[e]	Aqueous aspirate Vitreous aspirate, washing or biopsy	Inoculated slants and smear are transported directly to the laboratory[e], RT, immediately Washing sent to laboratory, RT, 2 h
Fungal[f]			
Aspergillus spp *Fusarium* spp Dematiaceous fungi *Scedosporium* spp *Candida albicans* *Candida glabrata* Other *Candida* spp	Calcofluor-KOH stain[g] Fungal culture (with bedside inoculation of culture plate)[g]	Aqueous aspirate Vitreous aspirate, washing or biopsy	Inoculated plate and smear are transported directly to the laboratory[g], RT, immediately Washing sent to laboratory, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; BCYE, buffered charcoal yeast extract; KOH, potassium hydroxide; RT, room temperature.

[a]Among the long list of bacterial causes of endophthalmitis, *Streptococcus agalactiae*, *Listeria monocytogenes*, and *Neisseria meningitidis* occur almost exclusively as a result of endogenous seeding of the eye. The other bacteria listed may cause endophthalmitis either secondary to trauma or surgery or following hematogenous seeding.

[b]Culture plates, including a sheep blood agar plate and a chocolate agar plate, should be inoculated directly at the patient's bedside at the time corneal scrapings are obtained (see footnote "b" for Table 12). If sufficient sample is available, a smear on a glass slide may also be prepared at the patient's bedside after plates are inoculated. The inoculated plates and slide (if prepared) are then transported directly to the microbiology laboratory.

[c]The laboratory should be notified when *Nocardia* spp is suspected so that special media can be used and routine culture plates will be incubated for up to 7 days.

[d]The most common *Mycobacterium* sp recovered from intraocular infections is *M. chelonae*, and this occurs almost exclusively as a complication of surgical procedures.

[e]Acid-fast smears and mycobacterial cultures should be performed in all postsurgical infections of the eye. A 7H-11 agar or a Lowenstein-Jensen agar slant should be inoculated at the patient's bedside. If sufficient clinical sample remains, a smear should be prepared. Both the slant and the smear (if prepared) should be transported directly to the laboratory for further processing. If after inoculating a slant and preparing a smear at the bedside, there is still unused specimen remaining, it should be transported in a sterile container immediately to the laboratory at RT for inoculation into broth media and subsequent instrument-based processing.

[f]Among the fungi listed, *Candida albicans*, *Candida glabrata*, and other *Candida* spp cause endogenous endophthalmitis as a result of hematogenous seeding of the eye. The other fungi listed typically cause infection following traumatic inoculation of the eye.

[g]At least one culture plate or slant containing a nonselective fungal growth medium should be inoculated directly at the patient's bedside at the time corneal scrapings are obtained. If sufficient sample is available, a smear on a glass slide may also be prepared at the patient's bedside after plates/slants have been inoculated. The inoculated plates/slants and slide (if prepared) are then transported directly to the microbiology laboratory. The smear is stained with Calcofluor-KOH in the laboratory and examined for fungal elements.

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

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].

IV. SOFT TISSUE INFECTIONS OF THE HEAD AND NECK

Infection of various spaces and tissues that occur in the head and neck can be divided into those arising from odontogenic, oropharyngeal, or exogenous sources. Odontogenic infections are caused commonly by endogenous periodontal or gingival flora [77]. These infections include peritonsillar and pharyngeal abscesses; deep space abscesses, such as those of the retropharyngeal, parapharyngeal, submandibular, and sublingual spaces; and cervical lymphadenitis [78, 79]. Complications of odontogenic infection can occur by hematogenous spread or by direct extension resulting in septic jugular vein thrombophlebitis (Lemierre syndrome), bacterial endocarditis, intracranial abscess, or acute mediastinitis [80, 81]. Accurate etiologic diagnosis depends upon collection of an aspirate or biopsy of inflammatory material from affected tissues and tissue spaces while avoiding contamination with mucosal microbiota. The specimen should be placed into an anaerobic transport container to support the recovery of anaerobic bacteria (both aerobic and facultative bacteria survive in anaerobic transport). Requests for Gram-stained smears are standard for all anaerobic cultures because they allow the laboratorian to evaluate the adequacy of the specimen by identifying inflammatory cells, provide an early presumptive etiologic diagnosis, and identify morphologic patterns indicative of mixed aerobic and anaerobic infections [82]. Additionally, spirochetes (often involved in odontogenic infection) cannot be recovered in routine anaerobic cultures but will be seen in the stained smear.

Infections caused by oropharyngeal flora include epiglottitis, mastoiditis, inflammation of salivary tissue, and suppurative parotitis [77, 83]. Because the epiglottis may swell dramatically during epiglottitis, there is a chance of sudden occlusion of the trachea if the epiglottis is disturbed, such as by an attempt to collect a swab specimen. Blood cultures are the preferred sample for the diagnosis of epiglottitis; if swabbing is attempted, it should be in a setting with available appropriate emergency response. Oropharyngeal flora also can extend into tissues of the middle ear, mastoid, and nasal sinuses, causing acute infection [77, 84]. Aspirated material, saline lavage of a closed space, and tissue or tissue scrapings are preferred specimens and must be transported in a sterile container. Tissues should be transported under sterile conditions and kept moist by adding a few drops of sterile, nonbacteriostatic saline. Although rarely implicated, if anaerobic bacterial pathogens are suspected, anaerobic transport is required. Note that filamentous fungi are common causes of chronic sinusitis, and they may not be recovered on swabs, even those obtained endoscopically. Endoscopic aspirates or tissue scrapings are the specimens of choice. For microbiology analysis, it is always best to submit the actual specimen, not a swab of the specimen.

Infections caused by exogenous pathogens (not part of the oral flora) include malignant otitis externa, mastoiditis, animal bites and trauma, irradiation burns, and complications of surgical procedures [84, 85]. Mucosal flora may play an etiologic role in these infections, most frequently gram-negative bacilli and staphylococci.

Key points for the laboratory diagnosis of head and neck soft tissue infections:

- A swab is not the specimen of choice for these specimens. Submit tissue, fluid, or aspirate when possible.
- Resist swabbing in cases of epiglottitis.
- Use anaerobic transport containers if anaerobes are suspected.
- Keep tissue specimens moist during transport.

The following tables include the most common soft tissue and tissue space infections of the head and neck that originate from odontogenic, oropharyngeal, and exogenous sources. The optimum approach to establishing an etiologic diagnosis of each condition is provided.

A. Infections of the Oral Cavity and Adjacent Spaces and Tissues Caused by Odontogenic and Oropharyngeal Flora (Table 14)

B. Mastoiditis and Malignant Otitis Externa Caused by Oropharyngeal and Exogenous Pathogens (Table 15)

V. UPPER RESPIRATORY TRACT BACTERIAL AND FUNGAL INFECTIONS

Infections in the upper respiratory tract usually involve the ears, the mucus membranes lining the nose and throat above the epiglottis, and the sinuses. Most infections involving the nose and throat are caused by viruses (see Section XIV for testing information). Inappropriate utilization of antibiotics for viral infections is a major driver of increasing antibiotic resistance. Proper diagnosis of infectious syndromes in this environment must involve laboratory tests to determine the etiology and thus inform the proper therapy.

Key points for the laboratory diagnosis of upper respiratory tract infections:

- Swabs are not recommended for otitis media or sinusitis. Submit an aspirate for culture.
- Most cases of otitis media can be diagnosed clinically and treated without culture support.
- Throat specimens require a firm, thorough sampling of the throat and tonsils, avoiding cheeks, gums, and teeth.
- *Haemophilus influenzae*, *Staphylococcus aureus*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* are not etiologic agents of acute pharyngitis and should not be identified or reported in throat cultures.
- Nasopharyngeal cultures cannot accurately predict the etiologic agent of sinusitis.

A. Otitis Media

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

Table 14. Laboratory Diagnosis of Infections of the Oral Cavity and Adjacent Spaces and Tissues Caused by Odontogenic and Oropharyngeal Flora

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Vincent angina (acute necrotizing ulcerative gingivitis)			
Mixed infection due to *Fusobacterium* spp and commensal *Borrelia* spp of the oral cavity	Gram stain; culture not recommended	Biopsy or irrigation and aspiration of lesion; swab not recommended	Sterile container, RT, immediately. If culture attempted, anaerobic transport vial, RT, 2 h
Epiglottitis and supraglottitis			
Normal host			
Haemophilus influenzae *Streptococcus pneumoniae* β-hemolytic streptococci *Staphylococcus aureus* *Neisseria meningitidis*	Gram stain Aerobic bacterial culture Blood cultures	Clinical diagnosis may not require specimen Swab of epiglottis[a] only if necessary Blood, 2–4 sets	Swab transport device, RT, 2 h Aerobic blood culture bottle, RT, immediately
Immunocompromised host			
Same bacteria as in the normal host above but also other agents such as *Pasteurella multocida*	Gram stain Aerobic bacterial culture Blood cultures	Clinical diagnosis may not require specimen Swab of epiglottis[a] only if necessary Blood, 2–4 sets	Swab transport device, RT, 2 h Aerobic blood culture bottle, RT, immediately
Aspergillus spp Other filamentous fungi	Calcofluor-KOH stain Fungal culture Fungal blood cultures	Biopsy or protected specimen brush Swab much less likely to recover fungi Blood, 2–4 sets	Sterile container, RT, 2 h Aerobic blood culture bottle formulated for fungi, RT, immediately, or lysis-centrifugation blood culture tubes, RT, immediately
Peritonsillar abscess			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus* (*milleri*) group *Arcanobacterium haemolyticum* Mixed aerobic and anaerobic bacterial flora of the oral cavity	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile anaerobic container, RT, immediately
Lemierre syndrome (internal jugular thrombophlebitis)			
Fusobacterium necrophorum Occasionally mixed anaerobic bacterial flora of the oral cavity including *Prevotella* spp and anaerobic gram-positive cocci	Gram stain Aerobic and anaerobic bacterial culture Blood cultures[b]	Biopsy, aspiration or irrigation of lesion; swab not recommended Blood, 2–4 sets	Sterile anaerobic container, RT, immediately Aerobic and anaerobic blood culture bottle, RT, immediately
Submandibular, retropharyngeal, and other deep space infections including Ludwig angina			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus (milleri*) group *Actinomyces* spp Mixed aerobic and anaerobic bacterial flora of the oral cavity	Gram stain Aerobic and anaerobic bacterial culture Blood cultures[b]	Biopsy, aspiration or irrigation of lesion; swab not recommended Blood, 2–4 sets	Sterile anaerobic container, RT, immediately Aerobic and anaerobic blood culture bottle, RT, immediately
Cervical lymphadenitis			
Acute infection			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus (milleri)* group Mixed aerobic and anaerobic bacterial flora of the oral cavity	Gram stain Aerobic and anaerobic bacterial culture Blood cultures[b]	Biopsy, aspiration or irrigation of abscess; swab not recommended Blood, 2–4 sets	Sterile anaerobic container, RT, immediately Aerobic and anaerobic blood culture bottle, RT, immediately
Chronic infection			
Mycobacterium avium complex *Mycobacterium tuberculosis* Other mycobacteria	Acid-fast smear AFB culture	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, 2 h
Listeria monocytogenes	Gram stain Aerobic and anaerobic bacterial culture Blood cultures[b]	Biopsy, aspiration or irrigation of abscess; swab not recommended Blood, 2–4 sets	Sterile container, RT, immediately Aerobic and anaerobic blood culture bottle, RT, immediately
Bartonella henselae	*Bartonella* NAAT[c] *Bartonella* culture[d] Histopathology (Warthin-Starry and H&E stains)	5 mL plasma Biopsy tissue Biopsy, aspiration or irrigation of abscess; swab not recommended Tissue in formalin for histopathology	EDTA tube, RT, 2 h Sterile container, RT, immediately Sterile container, RT, immediately Container for pathology, indefinite

Abbreviations: AFB, acid-fast bacilli; EDTA, ethylenediaminetetraacetic acid; H&E, hematoxylin and eosin; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

[a]Alert! Consider risk. During specimen collection, airway compromise may occur, necessitating the availability of intubation and resuscitation equipment and personnel.

[b]Blood cultures should be performed at the discretion of the healthcare provider.

[c]Note that nucleic acid tests are not usually available locally and must be sent to a reference laboratory with a resulting longer turnaround time.

[d]The laboratory should be alerted if *Bartonella* cultures will be requested so that appropriate media are available at the time the specimen arrives in the laboratory; even then, the yield of *Bartonella* culture is very low. When available, *Bartonella* nucleic acid testing is more sensitive. A portion of the specimen should be sent to the histopathology laboratory for H&E and Warthin-Starry stains.

Table 15. Laboratory Diagnosis of Mastoiditis and Malignant Otitis Externa Caused by Oropharyngeal and Exogenous Pathogens

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Mastoiditis			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Streptococcus pyogenes* *Staphylococcus aureus* *Pseudomonas aeruginosa* Enterobacteriaceae Anaerobic bacteria	Gram stain Aerobic and anaerobic bacterial culture	Middle ear fluid obtained by tympanocentesis or biopsy of mastoid tissue; swab not recommended	Sterile anaerobic container, RT, immediately
Mycobacterium tuberculosis	Acid-fast smear AFB culture	Biopsy of mastoid tissue	Sterile container, RT, 2 h
Malignant otitis externa			
Pseudomonas aeruginosa	Gram stain Aerobic bacterial culture	Scraping or fluid from external canal or tissue biopsy from temporal bone or mastoid	Sterile container, RT, 2 h

Abbreviations: AFB, acid-fast bacilli; RT, room temperature.

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

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

Table 16. Laboratory Diagnosis of Otitis Media

Etiological Agents[a]	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Streptococcus pyogenes* *Pseudomonas aeruginosa* *Alloiococcus otitidis* *Staphylococcus aureus*	Gram stain, Aerobic bacterial culture	Tympanocentesis fluid Mini-tipped swab of fluid draining from the middle ear cavity in patients with myringotomy tubes or otorrhea	Sterile container, RT, <2 h Swab transport device, RT, 2 h

Abbreviation: RT, room temperature.

[a]Viruses are often the etiologic agent, but microbiologic studies do not help with treatment decisions.

Table 17. Laboratory Diagnosis of Sinusitis

Etiological Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Acute maxillary sinusitis			
Bacterial			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Staphylococcus aureus*[a] *Streptococcus pyogenes*[a]	Gram stain Aerobic bacterial culture	Aspirate obtained by antral puncture	Sinus secretion collector (vacuum aspirator) Sterile container, RT, <2 h
		Middle meatal swab specimen obtained with endoscopic guidance (in adults)	Swab transport device, RT, 2 h
Complicated (chronic) sinusitis			
Bacterial			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Staphylococcus aureus* *Streptococcus pyogenes* *Pseudomonas aeruginosa* Enterobacteriaceae Mixed aerobic-anaerobic flora from the oral cavity	Gram stain Aerobic and anaerobic bacterial culture	Aspirate obtained by antral puncture[b]	Sinus secretion collector (vacuum aspirator) Sterile anaerobic container, RT, <2 h[c]
		Tissue or aspirate obtained surgically	Sterile anaerobic container, RT, <2 h[c]
Fungal			
Aspergillus spp Mucormycetes *Fusarium* spp Other molds	Calcofluor-KOH stain Fungus culture	Aspirate obtained by antral puncture[b]	Sinus secretion collector (vacuum aspirator) Sterile aerobic container, RT,<2 h
		Tissue or aspirate obtained surgically	Sterile aerobic container, RT, <2 h

Abbreviations: KOH, potassium hydroxide; RT, room temperature.

[a] *Staphylococcus aureus* and *Streptococcus pyogenes* do cause acute maxillary sinusitis but only infrequently.

[b] Antral puncture is a useful method for sampling the maxillary sinuses.

[c] Anaerobic transport vials are good for both aerobic and anaerobic bacteria.

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

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,

Table 18. Laboratory Diagnosis of Pharyngitis

Etiological Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Streptococcus pyogenes	Rapid direct antigen test (followed by a culture or NAAT test if negative)[a]	Dual pharyngeal swab	Swab transport device, RT, <2 h
	Direct NAAT[b]	Pharyngeal swab	Swab transport device, RT, <2 h
	Nucleic acid probe tests[b]	Pharyngeal swab	Stability as specified by lab or manufacturer
			Swab transport device, RT, <2 h if reflex culture is to be performed
Groups C and G β-hemolytic streptococci[c]	Throat culture and antigen tests on isolates for groups C and G streptococci	Pharyngeal swab	Swab transport device, RT, <2 h
	NAAT	Follow manufacturer's instructions for the method used	
Arcanobacterium haemolyticum[d]	Throat culture for *A. haemolyticum*	Pharyngeal swab	Swab transport device, RT, <2 h
Neisseria gonorrhoeae[d]	Throat culture or NAAT[e] for *N. gonorrhoeae*	Pharyngeal swab	Swab transport device, RT, <2 h
Corynebacterium diphtheriae[d]	Methylene blue stain *C. diphtheriae* culture	Pseudomembrane	Sterile container, RT, <2 h
Fusobacterium necrophorum	Anaerobic incubation. A selective medium is available	Pharyngeal swab	Anaerobic swab transport, RT, <2 h
Viral			
EBV	Monospot test[f] EBV serology	5 mL serum	Clot tube, RT, <2 h or refrigerate <24 h
HSV (usually type 1)	Direct detection test[g] Culture[g]	Swab of pharyngeal lesion	Swab transport device, RT, <2 h
	HSV IgG and IgM serology[h]	5 mL serum	Clot tube, RT, <2 h or refrigerate <24 h
CMV	CMV IgM serology	5 mL serum	Clot tube, RT, <2 h or refrigerate <24 h
HIV	See Section XIV		

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

[a]A rapid antigen test for *Streptococcus pyogenes* may be performed at the point of care by healthcare personnel or transported to the laboratory for performance of the test. There are numerous commercially available direct antigen tests. These vary in terms of sensitivity and ease of use; the specific test employed will dictate the swab transport system used. In pediatric patients, if the direct antigen test is negative, and if the direct antigen test is known to have a sensitivity of <80%, a second throat swab should be examined by a more sensitive direct NAAT or by culture as a means of arbitrating possible false-negative direct antigen test results [102]. This secondary testing is not necessarily required in adults [103]. A convenient means of facilitating this 2-step algorithm of testing for *Streptococcus pyogenes* in pediatric patients is to collect a dual swab initially, recognizing that the second swab will be discarded if the direct antigen test is positive.

[b]Direct and amplified NAATs for *Streptococcus pyogenes* are more sensitive than direct antigen tests and, as a result, negative direct NAAT results do not have to be arbitrated by a secondary test. The swab transport device should be compatible with the NAAT used. Direct nucleic acid probe tests are usually performed on enriched broth cultures, thus requiring longer turnaround times. Some amplified tests for point-of-care use are quite rapid.

[c]Detection of group C and G β-hemolytic streptococci is accomplished by throat culture in those patients in whom there exists a concern for an etiologic role for these organisms. Only large colony types are identified, as tiny colonies demonstrating groups C and G antigens are in the *Streptococcus anginosus* (*S. milleri*) group. Check with the laboratory to determine if these are routinely looked for.

[d]*Arcanobacterium haemolyticum*, *Neisseria gonorrhoeae*, and *Corynebacterium diphtheria* cause pharyngitis only in limited epidemiologic settings. The laboratory will not routinely recover these organisms from throat swab specimens. If a clinical suspicion exists for one of these pathogens, the laboratory should be notified so that appropriate measures can be applied.

[e]Use of NAAT for detecting gonorrhea in throat specimens is currently an off-label use of this test but may be validated for use in the laboratory.

[f]If the Monospot test is positive, it may be considered diagnostic for EBV infection. Up to 10% of Monospot tests are, however, falsely negative. False-negative Monospot tests are encountered most often in younger children but may occur at any age. In a patient with a strong clinical suspicion for EBV infection and a negative Monospot test, a definitive diagnosis can be achieved with EBV-specific serologic testing. Such testing can be performed on the same sample that yielded a negative Monospot test. Alternatively, the Monospot test can be repeated on a serum specimen obtained 7–10 days later, at which time, if the patient had EBV infection, the Monospot is more likely to be positive. See Section XIV on viral diagnostics.

[g]Probable cause of pharyngitis only in immunocompromised patients. Numerous rapid tests based on detecting HSV-specific antigen (by direct fluorescent antibody) directly in clinical material have been developed; the nonspecific stain Tzanck test is very insensitive and is not recommended. A swab should be used to aggressively collect material from the base of multiple pharyngeal lesions, and then placed in a swab transport device which is compatible with the test to be performed. Culture may be useful in immunocompromised patients.

[h]HSV serology is useful primarily for immunostatus and exposure status testing. IgM serology is no longer recommended.

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].

VI. LOWER RESPIRATORY TRACT INFECTIONS

Respiratory tract infections are among the most common infectious diseases. The list of causative agents continues to expand as new pathogens and syndromes are recognized. This section describes the major etiologic agents and the microbiologic approaches to the diagnosis of bronchitis and bronchiolitis; community-acquired pneumonia (CAP); hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP); infections of the pleural space; bronchopulmonary infections in patients with cystic fibrosis; and pneumonia in the immunocompromised host. The reader is referred to various practice guidelines that have been written in recent years by the Infectious Diseases Society of America, the American Thoracic Society, and the American Society for Microbiology, among other clinical practice groups that describe the clinical features, diagnostic approaches, and patient management aspects of many of these syndromes.

The table below summarizes some important caveats when obtaining specimens for the diagnosis of respiratory infections.

Key points for the laboratory diagnosis of lower respiratory tract infections:

- NAATs have largely replaced rapid antigen tests and culture for respiratory virus detection.
- The laboratory should be contacted for specific instructions prior to collection of specimens for fastidious pathogens such as *Bordetella pertussis*.
- First morning expectorated sputum is always best for bacterial culture.
- Blood cultures that accompany sputum specimens may occasionally be helpful, particularly in high-risk patients with CAP.
- The range of pathogens causing exacerbations of lung disease in cystic fibrosis patients has expanded, and specimens for mycobacterial and fungal cultures should be collected in some patients.
- In the immunocompromised host, a broad diagnostic approach based upon invasively obtained specimens is suggested.
- Bronchoscopy with washings is the optimal diagnostic specimen in pediatrics.

A. Bronchitis and Bronchiolitis

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).

Table 19. Laboratory Diagnosis of Bronchiolitis, Bronchitis, and Pertussis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bronchiolitis			
Viruses[a]			
RSV Rhinovirus Adenovirus Coronavirus HMPV Enterovirus PIV Influenza virus Human bocavirus type I	NAAT[b] Rapid antigen detection tests[c] Virus culture[d]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs NP swabs or aspirates, nasal washes NP swabs, NP aspirates, nasal washes, throat washes or swabs	VTM or sterile container (washes, etc), transport RT, < 2 h or refrigerated (2°C–8°C), 24–48 h VTM or sterile container (washes, etc) transport RT <2 h or refrigerated (2°C–8°C, 24–48 h
Acute bronchitis			
Bacteria			
Mycoplasma pneumoniae	NAAT[e]	Throat swab[c], NP swab, NP aspirates	NP swab, aspirate or wash Suitable transport device, RT, 2 h
	Mycoplasma IgG and IgM serology (EIA)	5 mL serum	Clot tube, RT, 2 h
Chlamydia pneumoniae	NAAT[e]	NP swab	Suitable transport device, RT, 2 h
	Chlamydia IgG and IgM serology (MIF)	5 mL serum	Clot tube, RT, 2 h
Viruses			
Influenza viruses PIV RSV HMPV Coronavirus Adenovirus Rhinovirus	NAAT[b] Rapid antigen detection tests[c] Virus culture[d]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	VTM or sterile container (washes, etc, transport RT <2 h or refrigerated (2°C–8°C), 24–48 h VTM or sterile container (washes, etc, transport RT <2 h or refrigerated (2°C–8°C, 24–48 h
Acute exacerbation of chronic bronchitis			
Bacteria			
Haemophilus influenzae (nontypeable) *Moraxella catarrhalis*	Gram stain Aerobic bacterial culture	Expectorated sputum	Sterile container, RT, 2 h or >2–24 h, 2°C–8°C
Chlamydia pneumoniae	See above under Acute bronchitis	See *Chlamydia and Mycoplasma* above	See above
Mycoplasma pneumoniae	See above under Acute bronchitis	See *Chlamydia* and *Mycoplasma* above	See above
Streptococcus pneumoniae	Gram stain Aerobic bacterial culture		
	Urine antigen[f]	First voided clean-catch urine specimen	Sterile container, RT, 2 h
Pseudomonas aeruginosa	Gram stain Aerobic bacterial culture	Expectorated sputum	Sterile container, RT, 2 h or >2–24 h, 2°C–8°C
Viruses			
Rhinovirus Coronavirus PIV (most often PIV3) Influenza virus RSV HMPV Adenoviruses	Rapid antigen detection tests[b] Virus culture[c] NAAT[d]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	VTM or sterile container (washes, etc, transport RT <2 h or refrigerated (2°C–8°C), 24–48 h
Bordetella pertussis			
	Bordetella culture on Regan-Lowe or Bordet-Gengou selective agar and NAAT	NP swabs; NP aspirates; nasal wash	For NAATs, use swabs tipped with polyester, rayon, nylon-flocked[g] For culture, use suitable transport[h], RT, ≤24 h

Abbreviations: EIA, enzyme immunoassay; HMPV, human metapneumovirus; IgG, immunoglobulin G; IgM, immunoglobulin M; MIF, microimmunofluorescent stain; NAAT, nucleic acid amplification test; NP, nasopharyngeal; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RT, room temperature; VTM, viral transport medium.

[a]Viruses are listed in decreasing order of frequency [110].

[b]Several US Food and Drug Administration (FDA)–cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Readers should check with their laboratory regarding availability and performance characteristics including certain limitations.

[c]Rapid antigen tests for respiratory virus detection lack sensitivity and depending upon the product, specificity. A recent meta-analysis of rapid influenza antigen tests showed a pooled sensitivity of 62.3% and a pooled specificity of 98.2% [130]. They should be considered as screening tests only. At a minimum, a negative result should be verified by another method. Specimen quality is critical to optimize these tests.

[d]Specimen type depends upon the virus that is sought. In general, throat swabs are at the least desirable. Care should be taken to preserve cells by using VTM or transporting specimens in a sterile container on wet ice as soon as possible after collection.

[e]There are several FDA-cleared assays available at this time. Two assays are comprehensive multiplex panels that contain *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* as part of a comprehensive respiratory syndromic panel. There is one singleplex assay for *M. pneumoniae*. Availability is laboratory specific. Clinicians should check with the laboratory for validated specimen sources, collection and transport, performance characteristics, and turnaround time. In general, avoid calcium alginate swabs and mini-tipped swabs for nucleic acid amplification tests.

[f]Sensitivity in nonbacteremic patients with pneumococcal pneumonia is 52%–78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80%–86%; specificity in adults is >90%. However, studies have reported a 21%–54% false-positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [128, 131].

[g]Cotton-tipped or calcium alginate swabs are not acceptable as they contain substances that inhibit polymerase chain reaction.

[h]Plating of specimens at the bedside is ideal but rarely done. Several types of transport media are acceptable. These include Casamino acid solution, Amies transport medium, and Regan-Lowe transport medium (Hardy Diagnostics) [132].

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

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

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

Table 20. Laboratory Diagnosis of Community-Acquired Pneumonia

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Streptococcus pneumoniae	Gram stain Culture	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
	Urine antigen[a]	Urine	Sterile container, RT, 24 h; >24 h–14 d, 2°C–8°C
Staphylococcus aureus *Haemophilus influenzae* Enterobacteriaceae *Pseudomonas aeruginosa*	Gram stain Culture	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
Legionella spp	Urine antigen *L. pneumophila* serogroup 1	Urine	Sterile container, RT, 24 h; >24 h–14 d, 2°C–8°C
	Selective culture on BCYE	Induced sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
	NAAT[b]	Induced sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
Mycoplasma pneumoniae	NAAT[b]	Throat swab, NP swab, sputum, BAL fluid	Transport in M4 media or other *Mycoplasma*-specific medium at RT or 4°C up to 48 h; ≥48 h, –70°C
	Serology IgM, IgG antibody detection	Serum	Clot tube, RT, 24 h; >24 h, 4°C
Chlamydia pneumoniae	NAAT[b]	NP swab, throat washings, sputum, bronchial specimens	Transport in M4 or other specialized transport medium at RT or 4°C up to 48 h; ≥48 h, –70°C
	Serology (MIF) IgM antibody titer; IgG on paired serum 2–3 wk apart	Serum	Clot tube, RT, 24 h; >24 h, 4°C
Mixed anaerobic bacteria (aspiration pneumonia)	Gram stain Aerobic and anaerobic culture	Bronchoscopy with protected specimen brush	Sterile tube with 1 mL of saline or thioglycollate; RT, 2 h; >2–24 h
		Pleural fluid (if available)	Sterile container, RT, without transport media ≤60 min; Anaerobic transport vial, RT, 72 h
Mycobacteria			
Mycobacterium tuberculosis and NTM	AFB smear AFB culture NAAT[c,d]	Expectorated sputum; induced sputum; bronchoscopically obtained specimens Gastric aspirates in pediatrics	Sterile container, RT, ≤2 h; ≤24 h, 4°C
Fungi			
Histoplasma capsulatum	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, <2 h; ≤24 h, 4°C
	Histology	Tissue	Sterile container, 4°C; formalin container, RT, 2–14 d
	Antigen tests	Serum, BAL, urine, pleural fluid (if available)	Clot tube, RT, 2 d; 2–14 d, 4°C Sterile container (urine), RT, 2 h; >2–72 h, 4°C
	Serum antibody (complement fixation)	Serum	Clot tube, RT, 24 h; 4°C, >24 h
Coccidioides immitis/posadasii	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens	Sterile container, RT, < 2 h; ≤24 h, 4°C
	Histology	Tissue	Formalin container, RT, 2–14 d; Sterile container, 2–14 d, 4°C
	Serum antibody IgM (ID, LA, EIA) IgG antibody (complement fixation, EIA)	Serum	Clot tube, RT, 24 h; >24 h, 4°C
Blastomyces dermatitidis	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, <2 h; ≤24 h, 4°C
	Histology	Tissue	Sterile container, 4°C, formalin container, RT, 2–14 d
	Antigen tests	Serum,	Clot tube, RT, 24 h
		Urine, BAL fluid, pleural fluid (if available)	Sterile container, 4°C, 2–14 d
	Serum antibody (complement fixation)	Serum	Clot tube, RT, 24 h; >24 h, 4°C

Table 20. Continued

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Viruses			
Influenza viruses A, B	Rapid antigen detection DFA Viral culture methods NAAT[c]	Nasal aspirates, nasal washes, NP swabs, throat washes, throat swabs, bronchoscopically obtained samples Transport in viral transport media, RT <2 h; 5 d, 4°C; >5 d, –70°C	
Adenovirus	DFA Viral culture methods NAAT[c]		
Parainfluenza viruses 1–4	DFA Viral culture methods NAAT[c]		
Respiratory syncytial virus	Rapid antigen detection DFA Viral culture methods NAAT[c]		
Human metapneumovirus	DFA NAAT[c]		
Coronaviruses	NAAT[c]		
Rhinovirus	Viral culture methods NAAT[c]		
Enteroviruses	Viral culture methods NAAT[c]		
Parasites			
Paragonimus westermani	Direct microscopic examination of pleural fluid and sputum for characteristic ova	Pleural fluid Sputum	Sterile container, fresh samples, 4°C, 60 min; preserved samples, RT, >60 min–30 d

Abbreviations: AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; DFA, direct fluorescent antibody test; EIA, enzyme immunoassay; ID, immunodiffusion; IgG, immunoglobulin G; IgM, immunoglobulin M; KOH, potassium hydroxide; LA, latex agglutination; MIF, microimmunofluorescent stain; NAAT, nucleic acid amplification test; NP, nasopharyngeal; NTM, nontuberculous mycobacteria; RT, room temperature.

[a]Sensitivity in nonbacteremic patients with pneumococcal pneumonia is 52%–78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80%–86%; specificity in adults is >90%. Not for use in children. Studies have reported a 21%–54% false-positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [128, 131].

[b]There are several US Food and Drug Administration (FDA)–cleared assays available at this time. Two assays are multiplex panels that contain *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* as part of a comprehensive respiratory syndromic panel. There is one singleplex assay for *M. pneumoniae*. Availability is laboratory specific. Clinicians should check with the laboratory for validated specimen sources, collection and transport, performance characteristics, and turnaround time. In general, avoid calcium alginate swabs and mini-tipped swabs for NAATs.

[c]Several FDA-cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Readers should check with their laboratory regarding availability and performance characteristics, including certain limitations.

[d]Sensitivity of NAAT with smear negative, culture positive is only 50%–80% (Updated Guidelines for Use of NAATs for Diagnosis of Tuberculosis. Morb Mortal Wkly Rept (MMWR) 2009; 58:7–10).

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, 10^6 colony-forming units (CFU)/mL; BAL fluid, 10^4 CFU/mL; protected specimen brush samples, 10^3 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

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

Table 21. Laboratory Diagnosis of Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia (Immunocompetent Host)

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Pseudomonas aeruginosa *Escherichia coli* *Klebsiella pneumoniae* *Enterobacter* spp *Serratia marcescens* *Acinetobacter* spp *Stenotrophomonas maltophilia* *Staphylococcus aureus* and MRSA *Haemophilus influenzae*	Blood culture Gram stain Quantitative or semi-quantitative aerobic and anaerobic culture[a]	Blood cultures Sputum Endotracheal aspirates BAL Protected specimen brush samples[a] Lung tissue	Routine blood culture bottles, RT <24 h Sterile cup or tube RT, 2 h; 4°C, >2–24 h
Streptococcus pneumoniae	As above plus urine antigen[b]	Urine	Sterile container RT, 24 h; >24 h–14 d, 2°C–8°C
Mixed anaerobes (aspiration)	Gram stain Culture[a]	Protected specimen brush samples[a] Lung tissue	Sterile tube with 1 mL of thioglycollate (for brush samples); Sterile container for tissue; RT, 2 h; 4°C, >2–24 h
Legionella spp	Culture on BCYE media NAAT[c]	Induced sputum Endotracheal aspirates BAL Protected specimen brush samples Lung tissue	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Urine antigen (*L. pneumophila* serogroup 1 only)	Urine	Sterile container RT, <24 h; 4°C >24 h–14 d
Fungi			
Aspergillus spp	Fungal stain—KOH with calcofluor; other fungal stains Fungal culture	Endotracheal aspirates BAL Protected specimen brush samples	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Histology	Lung tissue	Sterile cup; RT, 2 h; or formalin container, RT, 2–14 d
	Galactomannan[d] (1–3) β-D-glucan	Serum, BAL	Clot tube 4°C, ≤5 d; >5 d, –70°C Sterile cup or tube RT, 2 h; 4°C, >2–24 h
Viruses			
Influenza viruses A, B Parainfluenza viruses Adenovirus RSV	Rapid antigen detection DFA Viral culture methods NAAT[e]	Nasal washes, aspirates NP swabs Endotracheal aspirates BAL Protected specimen brush samples	Transport in viral transport media, RT or 4°C, 5 d; –70°C, >5 d

Abbreviations: BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; DFA, direct fluorescent antibody; KOH, potassium hydroxide; MRSA, methicillin-resistant *Staphylococcus aureus*; NAAT, nucleic acid amplification test; NP, nasopharyngeal; RSV, respiratory syncytial virus; RT, room temperature.

[a]Anaerobic culture should only be done if the specimen has been obtained with a protected brush or catheter and transported in an anaerobic transport container or by placing the brush in 1 mL of prereduced broth prior to transport.

[b]Sensitivity in nonbacteremic patients with pneumococcal pneumonia is 52%–78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80%–86%; specificity in adults is >90%. However, studies have reported a 21%–54% false-positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [128, 131].

[c]No US Food and Drug Administration (FDA)–cleared test is currently available. Availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics, and turnaround time.

[d]Performance characteristics of these tests are reviewed in references [133, 134].

[e]Several FDA-cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Readers should check with their laboratories regarding availability and performance characteristics, including certain limitations.

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

Table 22. Laboratory Diagnosis of Infections of the Pleural Space

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Aerobes			
Staphylococcus aureus	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
Streptococcus pneumoniae	As above, plus *S. pneumoniae* urinary antigen	Urine, pleural fluid	Sterile container, RT, 24 h; >24 h–14 d, 2°C –8°C; Sterile container, RT, 2 h; 4°C, >2–24 h
Streptococcus pyogenes *Streptococcus anginosus* group Enteric gram-negative bacilli *Enterococcus* spp *Pseudomonas aeruginosa*	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
Nocardia	Gram stain Modified acid-fast stain Culture (include selective BCYE or other selective media)		
Legionella	Gram stain—carbolfuchsin counter stain Culture on BCYE	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
	Legionella urinary antigen (*L. pneumophila* serogroup 1 only)	Urine	Sterile container, RT, <24 h; 4°C, >24 h–14 d
Anaerobes			
Bacteroides fragilis group *Prevotella* spp *Fusobacterium nucleatum* *Peptostreptococcus* *Actinomyces* spp	Gram stain Anaerobic culture	Pleural fluid	Anaerobic transport vial, RT, 72 h; without transport RT ≤60 min
Mycobacteria			
Mycobacterium tuberculosis	Acid-fast stain Mycobacterial culture NAAT[a]	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
	Histology	Pleural or lung biopsy Pleural fluid	Sterile container, RT, 2 h; 4°C, 3 d Formalin container, RT, 2–14 d
Fungi			
Fungi	Fungal stain—calcofluor-KOH; other fungal stains Fungal culture	Pleural fluid Pleural biopsy required for some diseases	Sterile container, RT, 2 h; 4°C, >2–24 h
Candida spp	As above plus may be evident on Gram stain	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
Aspergillus	General fungal assays (ie, stains, culture, serology) plus galactomannan, (1–3)-β-D-glucan[b]	BAL	Sterile container, 4°C, ≤5 d; –70°C >5 d
		Serum	Clot tube RT, 2 d; 4°C
Histoplasma capsulatum	Fungal stain—calcofluor-KOH; other fungal stains Fungal culture Histology	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
		Pleural biopsy	Sterile container, RT, 2 h; 4°C, >2–24 h; formalin container for histology, RT 2–14 d
	Antigen test[c]	Serum, urine, BAL, pleural fluid	Clot tube, RT, 2 d; 4°C, 2–14 d Sterile container (urine and fluid), RT 2 h; >2–72 h, 4°C
	Serum antibody (complement fixation)	Serum	Clot tube RT, 2 d; 4°C, 2–14 d
Coccidioides immitis/posadasii	General fungal assays (ie, stains, culture, serology) plus histology	Pleural fluid Pleural biopsy	Sterile container, RT, 2 h; 4°C, >2–24 h
	Serum antibody IgM (ID, LA, EIA) IgG antibody (complement fixation, EIA)	Serum	Clot tube, RT, 2 d; 4°C, 2–14 d
Blastomyces dermatitidis	Fungal stains and cultures (but not serology) plus histology	Pleural fluid Pleural biopsy	Sterile container, RT, 2 h; 4°C, >2–24 h
	Antigen test[c]	Urine, BAL, pleural fluid, serum	4°C, ≤5 d
Parasites			
Paragonimus westermani *Entamoeba histolytica* *Echinococcus* *Toxoplasma gondii*	Direct microscopic examination of pleural fluid and sputum for characteristic ova Direct examination of pleural fluid for troph and cyst forms Direct examination of pleural fluid for scolices Giemsa-stained smear of pleural fluid or pleural biopsy	Pleural fluid Sputum Pleural fluid	Sterile container, fresh samples 4°C, 60 min; RT, preserved samples >60 min–30 d

Abbreviations: BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; EIA, enzyme immunoassay; ID, immunodiffusion; IgG, immunoglobulin G; IgM, immunoglobulin M; KOH, potassium hydroxide; LA, latex agglutination; NAAT, nucleic acid amplification test; RT, room temperature.

[a]No US Food and Drug Administration–cleared test is currently available. Availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics and turnaround time.

[b]Performance characteristics of these tests are reviewed in references [133, 134].

[c]May cross-react with other endemic mycoses.

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

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.

Table 23. Laboratory Diagnosis of Pulmonary Infections in Cystic Fibrosis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Staphylococcus aureus *Haemophilus influenzae* *Streptococcus pneumoniae* Enteric bacilli *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia* *Achromobacter* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
Burkholderia cepacia complex	Culture using *B. cepacia* selective agar	Throat swabs[a], expectorated sputum; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Opportunistic glucose nonfermenting gram-negative rods *Burkholderia gladioli* *Inquilinus* spp *Ralstonia* spp *Cupriavidus* spp *Pandoraea* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
Mycobacterium spp			
Mycobacterium abscessus *Mycobacterium avium* complex	Mycobacterial culture Mycobacterial culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Fungi			
Aspergillus spp *Scedosporium* spp *Trichosporon*	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Viruses			
RSV Influenza Adenovirus Rhinovirus Coronavirus Parainfluenza virus Human metapneumovirus	Rapid antigen detection DFA Viral culture methods NAAT[b]	Nasal aspirates, nasal washes, NP swabs, throat washes, throat swabs; bronchoscopically obtained specimens	Transport in viral transport media, RT or 4°C, 5 d; –70°C, >5 d

Abbreviations: DFA, direct fluorescent antibody; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; NP, nasopharyngeal; RSV, respiratory syncytial virus; RT, room temperature.

[a]Young children < 8 years of age only; often called "gag sputum."

[b]Several US Food and Drug Administration–cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Readers should check with their laboratories regarding availability and performance characteristics, including certain limitations.

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

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.

VII. INFECTIONS OF THE GASTROINTESTINAL TRACT

Gastrointestinal infections include a wide variety of disease presentations as well as infectious agents. For many of these infections, particularly noninflammatory diarrhea and acute gastroenteritis of short duration, no laboratory testing is recommended [135]. This section addresses the laboratory approach to establishing an etiologic diagnosis of esophagitis, gastritis, gastroenteritis, and proctitis.

Key points for the laboratory diagnosis of gastrointestinal infections:

- The specimen of choice to diagnose diarrheal illness is the diarrheal stool, not a formed stool or a swab, with a notable exception in pediatrics where a swab is acceptable when feces is noted on the swab.
- Toxin or nucleic acid amplification testing for *C. difficile* should only be done on diarrheal stool, not formed stools, unless the physician notes that the patient has ileus.

Table 24. Laboratory Diagnosis of Pneumonia in the Immunocompromised Host

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
See list of bacterial agents responsible for CAP and HAP above	See Table 21	See Table 21	See Table 21
Additional bacterial pathogens of interest *Salmonella* (nontyphoidal) *Elizabethkingia meningoseptica* *Listeria monocytogenes*	Gram stain Culture	Expectorated sputum Bronchoscopically obtained specimens	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
Nocardia and other aerobic Actinomycetes	Gram stain Modified acid-fast stain Culture (include selective BCYE or other selective media)	Expectorated sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
Rhodococcus	Gram stain Culture		
Viruses			
Respiratory viruses	See Tables 20 and 21	See Tables 20 and 21	See Tables 20 and 21
Cytomegalovirus	Shell vial culture combined with antigen detection; use with cytologic analysis and/or tissue histology for interpretation	Expectorated sputum Bronchoscopically obtained specimens Lung tissue	Transport in VTM, 4°C, 5 d; –70°C, >5 d
	NAAT[a]	Plasma, BAL	Clot tube, RT, 30 min; 4°C, >30 min–24 h
	Quantitative antigenemia (losing favor vs NAAT)	Plasma	EDTA tube, RT, 6–8 h; 4°C, >8–24 h
Herpes simplex virus	Culture combined with antigen detection; Use with cytologic analysis and/or tissue histology for interpretation NAAT[a]	Histopathology of bronchoscopically obtained specimens (protected brush) Lung tissue	Transport in viral transport media, 4°C, 5 d; –70°C, >5 d
Mycobacterium spp			
M. tuberculosis	Acid-fast stain AFB culture NAAT Histology	Expectorated sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
M. avium intracellulare complex *M. kansasii* *M. xenopi* *M. haemophilum* Rapid growers, eg, *M. abscessus*	Acid-fast stain AFB culture	Expectorated sputum Bronchoscopically obtained specimens	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
	Histology	Lung tissue	Formalin container, RT, 2–14 d
Fungi			
Pneumocystis jirovecii[b]	DFA on BAL or sputum (not tissue) NAAT[a] Cytologic stains (liquid samples)	Expectorated sputum Induced sputum Bronchoscopically obtained specimens	Sterile cup or tube, RT, 2 h; 4°C, >2 h–7 d
	Tissue stains	Tissue	RT, 2 h; 4°C, >2–24 h Formalin container, RT, 2–14 d
Cryptococcus neoformans	Calcofluor or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
	Cryptococcal antigen test	Serum, 1 mL	Clot tube, RT, 1 h; 4°C, >1 h–7 d
	Tissue stains	Tissue	Formalin container, RT, 2–14 d Sterile container, RT, 2 h; 4°C, >2–24 h
Aspergillus spp	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Tissue	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
	Galactomannan (1–3)-β-D-glucan	Serum BAL[c]	Clot tube, 4°, ≤5 d; >5 d, –70°C Sterile container for BAL, RT, 2 h; 4°C, >2–24 h

Table 24. Continued

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Fusarium spp	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
	Histology/GMS stain	Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h Formalin container, RT, 2–14 d
	Fungal blood culture (see blood culture section)	Blood in aerobic blood culture bottle or lysis-centrifugation tube	RT, 4 h
Zygomycetes such as *Rhizopus*, *Mucor*, *Absidia* spp *Pseudoallescheria boydii*	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube, RT, 2 h; 4°C, >2–24 h
Histoplasma capsulatum	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container, RT, 2 h; 4°C, >2–24 h
	Fungal blood culture (see blood culture section)	Blood in aerobic blood culture bottle or lysis-centrifugation tube	RT, 4 h
	Antigen test	Serum, urine, BAL, pleural fluid (if applicable)	Clot tube for serum, RT, 2 d; 4°C, 2–14 d Sterile container for other samples, 4°C, ≤5 d
	Serology (complement fixation)	Serum	RT, 2 d; 4°C, 2–14 d
Coccidioides immitis/posadasii	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container, RT, 2 h; 4°C, >2–24 h
	Serum antibody IgM (ID, LA, EIA) IgG antibody (complement fixation, EIA)	Serum	Clot tube, RT, 2 d; 4°C, 2–14 d
Other endemic fungi	Calcofluor-KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container, RT, 2 h; 4°C, >2–24 h
	Antigen test (*Blastomyces*)	Serum, urine, BAL, pleural fluid (if applicable)	Clot tube for serum, RT, 2 d; 4°C, 2–14 d; Sterile container for other samples, 4°C, ≤5 d
Parasites			
Toxoplasma gondii	Microscopy—Giemsa stain smears (tissue) NAAT[a]	Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container, RT, 2 h; 4°C, >2–24 h
	IgM antibody detection	Serum	Clot tube, RT, 2 d; 4°C, 2–14 d
Enterocytozoon bieneusi (Microsporidiosis)	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d
	Modified trichrome stain NAAT	Induced sputum Bronchoscopically obtained specimens	Sterile container, RT, 2 h; 4°C, >2–24 h
Cryptosporidiosis	Modified acid-fast stain DFA NAAT[a]		
	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d
Strongyloides stercoralis	Microscopic wet mount examination of liquid samples for larval forms Culture (consult laboratory for availability)	Induced sputum Bronchoscopically obtained specimens	Sterile container, RT, 2 h; 4°C,>2–24 h
	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d

Abbreviations: AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; CAP, community-acquired pneumonia; DFA, direct fluorescent antibody test; EDTA, ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; GMS, Gamori methenamine silver stain; HAP, healthcare-associated pneumonia; ID, immunodiffusion; IgG, immunoglobulin G; IgM, immunoglobulin M; KOH, potassium hydroxide; LA, latex agglutination; NAAT, nucleic acid amplification test; RT, room temperature; VTM, viral transport medium.

[a]No US Food and Drug Administration (FDA)–cleared test is currently available for respiratory source and availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics, and turnaround time.

[b]In the appropriate clinical setting, an elevated serum or BAL β-D-glucan level is highly suggestive of *P. jirovecii* infection. A positive result should be followed by a definitive test for the organism, such as NAAT or DFA.

[c]Only galactomannan assays are FDA-cleared for this source.

A. Esophagitis

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

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.

C. Gastroenteritis, Infectious, and Toxin-Induced Diarrhea

Gastrointestinal infections encompass a wide variety of symptoms and recognized infectious agents (Table 27). The appropriate diagnostic approach to diarrheal illness is determined by the patient's age and status, severity of disease, duration and type of illness, time of year, and geographic location. Fecal testing using culture or culture-independent methods is indicated for severe, bloody, febrile, dysenteric, nosocomial, or persistent diarrheal illnesses [138]. Communication with the laboratory is required to determine what organisms, methods, and screening parameters are included as part of the routine enteric pathogen culture or culture-independent method. Most laboratories will have the ability to culture for *Salmonella*, *Shigella*, and *Campylobacter* and test for Shiga toxin–producing *Escherichia coli*. Culture independent methods are often routinely available for *Clostridium difficile* and, although available, may not be routinely employed for other bacterial and viral causes of gastrointestinal infections. Stool culture often fails to detect the causative agent and, when necessary, culture-independent methods are recommended as adjunct methods. The specimen of choice is the diarrheal stool (ie, takes the shape of the container). Multiple stool specimens are rarely indicated for the detection of stool pathogens. In studies of adult patients who submitted >1 specimen, the enteric pathogen was detected in the first sample 87%–94% of the time, with the second specimen bringing the positive rate up to 98% [139]. In pediatric patients, the first specimen detects 98% of the enteric pathogens [140]. Thus, one sample for children and a second for selected adult patients may be considered. Rectal swabs are less sensitive than stool specimens when culture methods are employed and are not recommended for culture from adults, but in symptomatic pediatric patients, rectal swabs and stool culture are equivalent in the ability to detect fecal pathogens [141, 142]. Rectal swabs have

Table 25. Laboratory Diagnosis of Esophagitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Candida spp	Calcofluor-KOH stain Fungus culture	Esophageal brushing or biopsy	Sterile container, RT, 2 h
	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2 h–14 d
Herpes simplex virus	HSV culture Direct fluorescent stain	Esophageal brushing or biopsy	Viral transport device, on ice, immediately
	NAAT	Esophageal brushing or biopsy	Closed container, RT, 2 h
	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2 h–14 d
Cytomegalovirus	CMV culture Direct fluorescent stain	Esophageal brushing or biopsy	Viral transport device, on ice, immediately
	NAAT	Esophageal brushing or biopsy Plasma	Closed container, RT, 2 h EDTA plasma
	Immunohistochemical stain	Esophageal biopsy	Formalin container, RT, 2 h–14 d

Abbreviations: CMV, cytomegalovirus; EDTA, ethylenediaminetetraacetic acid; HSV, herpes simplex virus; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

Table 26. Laboratory Diagnosis of Gastritis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Helicobacter pylori	*H. pylori* stool antigen test	Stool specimen	Closed container, RT, 2 h
	Urea breath test[a]	Radiolabeled breath	Special collection device
	Histopathological examination[b]	Same as above	Formalin container, RT, 2 h–14 d
	Agar-based or rapid tissue urease tests[c]	Same as above	Closed container, RT, 2 h
	Gram stain *H. pylori* culture[b]	Two biopsies from antrum and 2 biopsies from posterior corpus	Sterile container, RT, immediately

Abbreviation: RT, room temperature.

[a]The patient ingests a cocktail containing ^{13}C-labeled urea and 15–30 minutes later, a breath sample is obtained and analyzed for the presence of ^{13}C-labeled CO_2 as an indication of the presence of *H. pylori* in the stomach.

[b]Gram stain and culture of properly collected and transported biopsy specimens has a sensitivity of 95% as does histopathological examination, but is usually unavailable and considered inappropriate by some.

[c]Agar-based or rapid urease tests have a slightly lower sensitivity of 90%–95% but offer the advantage of providing rapid results. They may be performed at the point of care or in the laboratory. When these tests are performed on gastric fluid, orogastric brush, or "string" specimens, they have lower sensitivity than when performed on biopsy specimens.

been shown to be as sensitive as stool specimens when culture independent methods are employed, although no tests are FDA-cleared for their use.

Stool Culture

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

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

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

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

Table 27. Laboratory Diagnosis of Gastroenteritis, Infectious, and Toxin-Induced Diarrhea

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Clostridium difficile	NAAT	Stool	Closed container, RT, 2 h
	GDH antigen and toxin detection with or without discrepant results arbitrated by NAAT; or NAAT plus toxin performed as part of an algorithm	Stool	Closed container, RT, 2 h
Salmonella spp *Shigella* spp *Campylobacter* spp	NAAT Routine stool enteric pathogen culture[a]	Stool	Closed container, RT, 2 h[b]
EHEC (including *E. coli* O157:H7 and other Shiga toxin–producing *Escherichia coli*)	NAAT for Shiga toxin genes	Stool	Closed container, RT, 2 h[b]
	Shiga toxin immunoassay	Stool	Closed container, RT, 2 h[b]
	Culture for *E. coli* O157:H7[c]	Stool	Closed container, RT, 2 h[b]
Yersinia spp *Vibrio* spp *Plesiomonas* spp *E. coli* (enterotoxigenic, enteroinvasive, enteropathogenic, enteroaggregative)	NAAT[d]	Stool	Closed container, RT, 2 h[b]
Yersinia spp *Vibrio* spp *Aeromonas* spp *Plesiomonas* spp *Edwardsiella tarda* *Staphylococcus aureus* *E. coli* (enterotoxigenic, enteroinvasive, enteropathogenic, enteroaggregative)	Specialized stool cultures[e]	Stool	Closed container, RT, 2 h[b]
Bacillus cereus *Clostridium perfringens* *Staphylococcus aureus*	Specialized procedure for toxin detection[f]	Stool	Closed container, RT, 2 h
Clostridium botulinum	Mouse lethality assay[g] (usually performed at the state public health laboratory)	Stool, gastric contents, vomitus[h]	Closed container Store and transport specimens at 4°C; do not freeze
Parasites			
Entamoeba histolytica/dispar *Blastocystis hominis*[i] *Dientamoeba fragilis* *Balantidium coli* *Giardia lamblia* Nematodes including *Ascaris lumbricoides*, *Strongyloides stercoralis*[j], *Trichuris trichiura*, hookworms Cestodes (tapeworms) Trematodes	Ova and parasite examination including permanent stained smear	Stool	Stool not in fixative <1 h RT, 5% or 10% buffered formalin and modified PVA, SAF, or commercially available 1-vial system, 2–24 h
E. histolytica	*E. histolytica* sp–specific immunoassay	Stool	Stool not in fixative
	NAAT[d]		Cary-Blair transport, RT, 24 h
Giardia lamblia[k] *Cryptosporidium* spp[k]	EIA	Stool	Stool in fixative, 2–24 h
	DFA	Stool	Stool in fixative, 2–24h
	NAAT[d]		Cary-Blair transport, RT, 24 h
	Histologic examination with electron microscopy confirmation		Formalin container, RT, 2–14 d
Coccidia including *Cryptosporidium*[k], *Cyclospora*, *Isospora*	Modified acid-fast stain[l] performed on concentrated specimen	Stool	Stool not in fixative <1 h RT, 5 or 10% buffered formalin and modified PVA, SAF, or commercially available 1-vial system, 2–24 h
Cryptosporidium[d], *Cyclospora*	NAAT[d]	Stool	Cary-Blair transport, RT, 24 h
Microsporidia	Modified trichrome stain[l] performed on concentrated specimen	Stool	Stool not in fixative <1 h RT, 5 or 10% buffered formalin and modified PVA, SAF, or commercially available 1-vial system, 2–24 h
Enterobius vermicularis	Pinworm paddle or Scotch tape prep	Perianal area	RT, 2 h
Virus			
Astrovirus[m] Calicivirus[m] (norovirus, sapovirus) Enteric adenovirus Enterovirus/parechovirus[m,n] Rotavirus[m]	NAAT	Stool	Closed container, RT, 2 h

Table 27. Continued

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Rotavirus Enteric adenovirus	EIA[o]	Stool	Closed container, RT, 2 h
Enteric adenovirus[p] Enterovirus/parechovirus[n]	Viral culture	Stool	Viral transport medium, on ice, 2 h
Cytomegalovirus	Histopathological examination	Biopsy	Formalin container, RT, 2–14 d
	CMV culture	Biopsy	Sterile container, RT, immediately
	NAAT for viral load[q]	Plasma	EDTA plasma tube, RT, 2 h or 2°C–8°C for 24 h, frozen −20°C for longer storage
Calicivirus (norovirus, sapovirus)	Outbreak investigation performed by public health officials	Stool	Closed container, RT, 2 h

Abbreviations: CMV, cytomegalovirus; DFA, direct fluorescent immunoassay; EDTA, ethylenediaminetetraacetic acid; EHEC, enterohemorrhagic *Escherichia coli*; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test; PVA, polyvinyl alcohol; RT, room temperature; SAF, sodium acetate formalin transport.

[a]A routine stool culture in most laboratories is designed to detect *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, and *Escherichia coli* O157 or Shiga toxin–producing *E. coli*.

[b]If the specimen cannot be transported to the laboratory within 2 hours, then it should be placed in vial containing Cary-Blair transport medium and transported to the laboratory within 24 hours.

[c]It is recommended that laboratories routinely process stool specimens for the presence of Shiga toxin–producing strains of *E. coli* including O157:H7. However, in some settings, this testing may be done only on specific request.

[d]Available as part of some multiplex panels.

[e]Specialized cultures are required to detect these organisms in stool specimens. In many cases, such cultures are performed only in public health laboratories and only in the setting of an outbreak. The laboratory should be notified whenever there is a suspicion of infection due to one of these pathogens.

[f]*Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* cause diarrheal syndromes that are toxin mediated. An etiologic diagnosis is made by demonstration of toxin in stool. Toxin assays are either performed in public health laboratories or referred to laboratories specializing in such assays.

[g]Testing for *Clostridium botulinum* toxin is either performed in public health laboratories or referred to laboratories specializing in such testing. The toxin is lethal and special precautions are required for handling. Note that it is considered a bioterrorism agent and rapid sentinel laboratory reporting schemes must be followed. Immediate notification of a suspected case to the state health department is mandated. For this purpose, 24-hour hotlines are available.

[h]Implicated food materials may also be examined for *C. botulinum* toxin, but most hospital laboratories are not equipped for food analysis.

[i]The role of *Blastocystis hominis* as a pathogen remains controversial. In the absence of other pathogens, it may be important where symptoms persist. Reporting semi-quantitative results (rare, few, many) can help determine significance and is a College of American Pathologists accreditation requirement for participating laboratories.

[j]Detection of *Strongyloides* in immunocompromised patients may require the use of Baermann technique or agar plate culture.

[k]*Cryptosporidium* and *Giardia lamblia* testing is often offered and performed together as the primary parasitology examination. Further studies should follow if a travel history or clinical symptoms suggest parasitic disease.

[l]These stains may not be routinely available.

[m]Also available as part of some multiplex panels.

[n]Asymptomatic shedding is common.

[o]Norovirus antigen assays have limited sensitivity and specificity and are not recommended for clinical use.

[p]Enteric adenoviruses may not be recovered in routine viral culture.

[q]A negative viral load test does not necessarily rule out CMV disease. Gastrointestinal disease due to CMV may be present in patients with negative viral load.

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

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

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.

D. Proctitis

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).

VIII. INTRA-ABDOMINAL INFECTIONS

This section is designed to optimize the activities of the microbiology laboratory to achieve the best approach for the identification of microorganisms associated with peritonitis and intraperitoneal abscesses, hepatic and splenic abscesses, pancreatitis, and biliary tract infection. As molecular analyses begin to be used to define the microbiome of the gastrointestinal and genitourinary tract, contemporary culture protocols will surely evolve to accommodate new, emerging information. The future use of gene amplification and sequencing for identification of microorganisms in these infections will likely show that for every organism currently identified by culture, there will be several times that number that cannot be cultivated using current technologies. To remain focused on contemporary methods currently available in the diagnostic microbiology laboratory, the tables outline the most likely agents of each entity (Table 29) and how best to evaluate the situation with existing techniques (Table 30).

Table 28. Laboratory Diagnosis of Proctitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Neisseria gonorrhoeae	NAAT[a] Routine aerobic culture employing media for the recovery of *N. gonorrhoeae*	Rectal swab	Transport is manufacturer dependent (consult lab) Swab in Amies or Stuart transport medium, RT, 8 h
Neisseria gonorrhoeae *Chlamydia trachomatis*	NAAT[a]	Rectal swab	Transport is manufacturer dependent
Chlamydia trachomatis	NAAT[a] Direct immunofluorescent stain	Rectal swab	Transport is manufacturer dependent
Herpes simplex virus	NAAT Viral culture	Rectal swab	Viral transport medium, RT, 2 h, wet ice if >2 h for culture
Treponema pallidum	RPR or VDRL with confirmatory *Treponema pallidum*–specific test or syphilis IgG	Serum	Clot tube, RT, 2 h

Abbreviations: IgG, immunoglobulin G; NAAT, nucleic acid amplification test; RPR, rapid plasma reagin; RT, room temperature; VDRL, Venereal Disease Research Laboratory.

[a]This is not yet a US Food and Drug Administration–approved specimen source. Availability of testing on this sample type is laboratory specific based on individual laboratory validation. Provider needs to check with the laboratory for optimal specimen and turnaround time.

Factors to consider when collecting specimens for laboratory diagnosis of intra-abdominal infections:

Key points for the laboratory diagnosis of intra-abdominal infections:

- The laboratory needs the specimen, not a swab of the specimen. Sufficient quantity of specimen must be collected to allow the microbiology laboratory to perform all the necessary and requested tests.
- The specimen of choice for an abscess is a sample of the contents plus a sample of the wall of the abscess when possible.
- Pus alone may not reveal the etiologic agent since the PMNs may have destroyed morphological evidence of microbial invasion.
- While most molecular tests have excellent sensitivity, a *Mycobacterium tuberculosis* NAAT test should be an adjunct to a culture and never ordered alone. No current commercial methods are FDA-cleared for intra-abdominal specimens, so laboratories must have validated the test they use.
- If *M. tuberculosis* is present, it is usually a sign of disseminated disease that must be thoroughly investigated.

A. Spontaneous Bacterial Peritonitis and Ascites

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

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

Table 29. Etiologic Agents Involved in Intra-abdominal Infections

Infection	Enterobacteriaceae	Gram-negative, Oxidase-positive Rods	Gram-negative Nonfermenters	Gram-positive Cocci	Gram-positive Rods	Anaerobes	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	*Mycobacterium* spp	Yeast	Dimorphic fungi	Molds	Parasites	Viruses
Spontaneous bacterial peritonitis/ascites	X			X			X		X	X	X			
Secondary peritonitis	X	X		X		X	X		X	X			X	X
Tertiary peritonitis	X	X		X		X	X		X	X		X		
Peritoneal dialysis-associated peritonitis	X	X		X	X	X			X			X		
Lesions of the liver	X	X		X		X	X	X		X			X	
Infections of biliary tree	X			X		X			X				X	X
Splenic abscess	X	X	X	X	X				X	X		X		
Secondary pancreatic infections	X			X		X				X				

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

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].

Table 30. Specimen Management for Intra-abdominal Infections

Condition	Diagnostic Procedure	Optimum Specimen	Transport Issues and Optimal Transport Time
Spontaneous bacterial peritonitis/ ascites; secondary peritonitis; tertiary peritonitis; peritoneal dialysis-associated peritonitis	Aerobic and anaerobic[a] culture: Gram stain prior to culture	10-50 mL concentrated peritoneal fluid and Sample in blood culture bottle[a]	RT; if >1 h, 4°C
	Blood culture	2–3 sets blood culture bottles	RT, do not refrigerate
	AFB stain and culture *Mycobacterium* NAAT[b]	Peritoneal fluid, aspirate or tissue	RT <1 h or 4°C
	Fungal culture and KOH or calcofluor white microscopy	Peritoneal fluid, aspirate or tissue	RT <1 h or 4°C
	Microscopy for ova and parasites[c]	Stool, peritoneal fluid, bile, duodenal aspirate	Transport stool in parasite transport vial; others <1 h at RT
Space-occupying lesions of the liver	Aerobic and anaerobic culture Gram stain specimen prior to culture	Lesion aspirate	Anaerobic transport; RT, if >1 h, 4°C
	Blood culture	2–3 sets in blood culture bottles	RT, do not refrigerate
	Cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*	Lesion aspirates *C. trachomatis* specimen may include swab of liver capsule or surrounding peritoneum	For *N. gonorrhoeae:* Amies charcoal transport, RT. For *C. trachomatis:* Chlamydia transport medium at 4°C
	NAAT *for N. gonorrhoeae* and *C. trachomatis*	Urethra, pelvic specimen (approved swabs), or urine (sterile cup)	RT for <1 h or 4°C
	Fungal culture and KOH or calcofluor white microscopy	10–50 mL fluid	RT, if >1 h, 4°C
	Serology for *Entamoeba histolytica/ dispar*	Serum	RT for <30 min, then 4°C. Freeze at –20°C if shipping to reference laboratory
	Antigen detection for *E. histolytica*	Liver aspirate	RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory
Infections of the biliary tree	Aerobic and anaerobic culture Gram stain before culture	Aspirate from lesion	Anaerobic transport device; RT, if >1 h, 4°C
	Blood culture	2–3 sets	RT; do not refrigerate
	AFB stain and culture	Fluid or tissue	≤1 h at RT or 4°C
	O&P exam	Stool, peritoneal fluid, bile, or duodenal aspirate	Closed container, RT, <2 h O&P transport vial, RT, 2–24 h
	Viral culture or NAAT	Aspirate or biopsy for CMV	Viral transport <1 h at RT. If >1 h, freeze (–70°C)
	Serology for *E. histolytica/dispar*	Serum	RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory
Splenic abscess	Aerobic and anaerobic culture Gram stain	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4°C
	Blood culture	2–3 sets	RT; do not refrigerate
	AFB stain and culture *Mycobacterium* NAAT can be done[b]	Fluid or tissue	RT. If >1 h, 4°C
	Fungal culture and KOH or calcofluor white microscopy	10–50 mL of aspirate or tissue	RT. If >1 h, 4°C
	Serology for *Entamoeba* and *Echinococcus*	Serum	RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory.
Secondary pancreatic infections	Aerobic and anaerobic culture Gram stain prior to culture	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4°C.
	Blood culture	2–3 sets	RT; do not refrigerate
	Fungal culture and KOH-calcofluor microscopy	10–50 mL aspirate or tissue	RT; if >1 h, 4°C

Abbreviations: AFB, acid-fast bacilli; CMV, cytomegalovirus; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; O&P, ova and parasite; RT, room temperature.

[a]If Gram stain reveals multiple morphologies of organisms, do not inoculate blood culture bottles with the fluid, as competitive bacterial growth could mask the recovery of clinically significant pathogens. If fluid is inoculated into blood culture bottles, a conventional culture must also be used. Anaerobic cultures of peritoneal fluid are only necessary in cases of secondary peritonitis.

[b]Depends on availability and should never substitute for culture because of variable sensitivity. Check with the microbiology laboratory for transport conditions. No commercial NAAT for mycobacteria available for nonrespiratory samples.

[c]Procedure to be used in cases of secondary peritonitis in appropriate clinical situations.

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

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

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

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

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

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.

IX. BONE AND JOINT INFECTIONS

Osteomyelitis arises from hematogenous seeding of bone from a distant site, extension into bone from a contiguous site, or direct inoculation of microorganisms into bone with surgery or trauma. Infections of native joints may also develop by any of these routes, with most occurring by hematogenous seeding. Infections of prosthetic joints are usually acquired from contamination at the time of arthroplasty implantation, but may occur due to subsequent hematogenous seeding or extension from contiguous sites.

The potential list of causative agents of bone and joint infections is diverse and largely predicated on the nature and pathogenesis of infection and the host. While bone and joint infections are usually monomicrobial, some may be polymicrobial.

Key points for the laboratory diagnosis of bone and joint infections:

- Swabs are not recommended for specimen collection, with aspirates and/or tissue biopsies being preferred.
- Blood cultures are indicated for detection of some agents of osteomyelitis and native joint infection, but not for routine prosthetic joint infection diagnosis.
- Joint fluids should ideally be cultured in blood culture bottles.
- For prosthetic joint infection diagnosis, 3–4 separate tissue samples should be submitted for culture; sonication of explanted prostheses may also be used to detect pathogens in biofilms.
- When anaerobic bacteria are suspected, which includes all cases of prosthetic joint infection, anaerobic transport containers should be used for transportation of tissues and fluids to the laboratory, and anaerobic cultures performed.
- Some agents of bone and joint infection are nonculturable or poorly culturable and require molecular and/or serologic methods for detection.

A. Osteomyelitis

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,

Table 31. Laboratory Diagnosis of Osteomyelitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Staphylococcus aureus Coagulase-negative staphylococci *Salmonella* spp[a] *Streptococcus* spp[b] *Enterococcus* spp Enterobacteriaceae *Candida* spp *Brucella* spp[c] *Pseudomonas* spp[d] Anaerobic bacteria	Gram stain Aerobic and anaerobic bacterial culture	Bone biopsy	Sterile anaerobic transport container, RT, 2 h
Kingella kingae	Aerobic bacterial culture *K. kingae* NAAT	Bone biopsy	Sterile container, RT, 2 h
Mycobacterium tuberculosis[e]	Acid-fast smear Mycobacterial culture *M. tuberculosis* NAAT[e]	Bone biopsy	Sterile container, RT, 2 h
Blastomyces spp *Coccidioides immitis/posadasii*	Calcofluor-KOH stain Fungus culture	Bone biopsy	Sterile container, RT, 2 h
Mixed aerobic and anaerobic bacterial flora of the oral cavity including *Actinomyces* spp in patients with maxillary or mandibular osteomyelitis[f]	Gram stain Aerobic and anaerobic bacterial culture	Bone biopsy	Sterile anaerobic transport container, RT, 2 h
Mixed bacterial flora in diabetic patients with skin and soft tissue extremity infections	Gram stain Aerobic and anaerobic bacterial culture	Bone biopsy	Sterile anaerobic transport container, RT, 2 h
Nocardia spp, other aerobic actinomycetes and soil filamentous fungi in patients with mycetoma[g]	Gram stain Aerobic bacterial culture *Nocardia* stain Calcofluor-KOH stain *Nocardia* culture Fungal culture	Bone biopsy	Sterile container, RT, 2 h

Abbreviations: KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

[a] *Salmonella* osteomyelitis occurs most often in patients with sickle cell trait or disease.

[b] *Streptococcus pneumoniae* osteomyelitis occurs most often in pediatric patients, not infrequently in the setting of pneumococcal bacteremia.

[c] *Brucella* spp will be recovered in standard aerobic bacterial cultures; however, the laboratory should be notified when *Brucella* spp is considered to be a potential cause of osteomyelitis so that cultures are examined only in a biological safety cabinet. Concomitant serologic testing is recommended.

[d] Hematogenous osteomyelitis caused by *Pseudomonas aeruginosa* and other *Pseudomonas* spp occurs most often in injection drug users. *P. aeruginosa* is the most common bacterial cause of calcaneal osteomyelitis in individuals who develop this infection after stepping on nails while wearing sneakers.

[e] The most common site of osteomyelitis due to *Mycobacterium tuberculosis* is the vertebral bodies. *M. tuberculosis* also represents one of the most common causes of clavicular osteomyelitis. Commercial NAATs are not US Food and Drug Administration–approved for nonrespiratory sites, so a laboratory-developed/validated test must be used if NAATs are requested.

[f] Chronic endodontic infections such as apical abscesses may extend into surrounding bone, resulting in osteomyelitis of the maxilla or mandible. These infections are caused by the aerobic and anaerobic bacterial flora of the oral cavity and may be either monomicrobial or polymicrobial. *Actinomyces* spp is a recognized pathogen in this setting; when *Actinomyces* spp is suspected, specimens should be transported to the laboratory under anaerobic conditions and cultures incubated for 10–14 days.

[g] Mycetoma is a chronic soft tissue infection of the extremities which can also extend into contiguous bone and connective tissue. It occurs most often in tropical and subtropical climates and may be characterized by the development of draining sinuses. Etiologic agents are derived from the soil. Sinus tract drainage material, when present, may be representative of the etiology of underlying osteomyelitis. In addition to the stains and cultures noted in the table, sinus drainage should also be examined grossly and microscopically for the presence of "sulfur granules" characteristic of this disease. Furthermore, the laboratory should be notified of the possibility of *Nocardia* spp as a pathogen so that appropriate media (eg, Middlebrook agar, Sabouraud dextrose agar, buffered charcoal yeast extract) can be inoculated which facilitate recovery of this organism.

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

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 >50 000 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

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.

X. URINARY TRACT INFECTIONS

Clinical microbiology tests of value in establishing an etiologic diagnosis of infections of the urinary tract are covered in this section, including specimens and laboratory procedures for the diagnosis of cystitis, pyelonephritis, prostatitis, epididymitis, and orchitis. Some special tests not available in smaller laboratories may be sent to a reference laboratory, but expect longer turnaround times for results.

Key points for the laboratory diagnosis of urinary tract infections (UTIs):

- Urine should not sit at room temperature for more than 30 minutes. Hold at refrigerator temperatures if not cultured

Table 32. Laboratory Diagnosis of Native Joint Infection and Bursitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Acute arthritis			
Staphylococcus aureus *Staphylococcus lugdunensis* *Streptococcus* spp Enterobacteriaceae *Pseudomonas* spp *Kingella kingae*[a] *Neisseria gonorrhoeae*[b]	Gram stain Aerobic and anaerobic bacterial culture *K. kingae* NAAT	Synovial fluid and/or synovium biopsy Blood	Sterile container, RT, 2 h Inoculate fluid into aerobic and anaerobic blood culture bottles Blood cultures
Brucella spp	*Brucella* serology *Brucella* culture	5 mL serum Synovial fluid and/or synovium biopsy Blood	Clot tube, RT, 2 h Sterile container, RT, 2 h Inoculate fluid into aerobic blood culture bottle Blood culture
Parvovirus B19	PV-B19 serology	5 mL serum	Clot tube, RT, 2 h
	PV-B19 NAAT	Synovial fluid	Closed container, RT, 2 h
Rubella	Rubella serology	5 mL serum	Clot tube, RT, 2 h
Subacute or chronic arthritis			
Chikungunya	Chikungunya serology	5 mL serum	Clot tube, RT, 2 h
Borrelia burgdorferi	Lyme serology	5 mL serum	Clot tube, RT, 2 h
	B. burgdorferi NAAT	Synovial fluid	Sterile container, RT, 2 h
	B. burgdorferi culture[c]	Synovial fluid	Closed container, RT, 2 h
Mycobacterium tuberculosis NTM	Acid-fast smear AFB culture *M. tuberculosis* NAAT[d]	Synovial fluid and/or synovium biopsy	Sterile container, RT, 2 h
Candida spp *Cryptococcus neoformans/gattii* *Blastomyces* spp *Coccidioides immitis/posadasii* *Aspergillus* spp	Calcofluor-KOH stain Fungal culture Serum cryptococcal antigen *Blastomyces dermatitidis* serology *Coccidioides immitis/ posadasii* serology	Synovial fluid and/or synovium biopsy 5 mL serum 5 mL serum 5 mL serum	Sterile container, RT, 2 h Clot tube, RT, 2 h Clot tube, RT, 2 h Clot tube, RT, 2 h
Septic bursitis			
Staphylococcus aureus	Gram stain Aerobic bacterial culture	Bursa fluid	Sterile container, RT, 2 h Inoculate fluid into aerobic and anaerobic blood culture bottles

Abbreviations: AFB, acid-fast bacilli; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; NTM, nontuberculous mycobacteria; PV, parvovirus; RT, room temperature.

[a] *Kingella kingae* is the most common cause of septic joint infections before the age of 4 years.

[b] *Neisseria gonorrhoeae* cultures of synovial fluid may be negative. NAATs for *N. gonorrhoeae* should be performed on genitourinary sites and/or freshly voided urine and, if clinically indicated, rectal and oropharyngeal swabs; culture for *N. gonorrhoeae* should be performed on specimens from genitourinary sites and, if clinically indicated, rectal and oropharyngeal swabs.

[c] Serology would be expected to be positive in the case of a positive NAAT or culture. Culture for *Borrelia burgdorferi* requires use of specialized media, rarely results in recovery of the organism, and is seldom done except in research settings.

[d] Detection of *Mycobacterium tuberculosis* or other *Mycobacterium* spp by microscopy or culture is very uncommon from synovial fluid in patients with joint infections due to these organisms. Analysis of synovial tissue enhances the likelihood of detection.

within 30 minutes, or use a urine transport device (boric acid or other preservative).

- Reflexing to culture after a positive pyuria screen should be a locally approved policy.
- The presence of 3 or more species of bacteria in a urine specimen usually indicates contamination at the time of collection, and interpretation is fraught with error.
- Do not ask the laboratory to report “everything that grows” without first consulting with the laboratory and providing documentation for interpretive criteria for culture that is not in the laboratory procedure manual.

A. Urinary Tract Infection/Pyelonephritis

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

Table 33. Laboratory Diagnosis of Prosthetic Joint Infection

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Staphylococcus aureus Coagulase-negative staphylococci *Enterococcus* spp *Streptococcus* spp Enterobacteriaceae *Pseudomonas aeruginosa* *Corynebacterium* spp *Cutibacterium (Propionibacterium) acnes* *Finegoldia magna* Other aerobic or anaerobic bacteria Fungi Mycobacteria	Aerobic and anaerobic bacterial culture (incubate anaerobic cultures up to 14 d for recovery of *C. acnes*) Gram stain not useful	Synovial fluid Multiple tissue biopsy samples Consider submitting prosthesis (if removed) for vortexing/sonication with aerobic and anaerobic culture of sonicated fluid	Sterile anaerobic transport container, RT, 2 h

Abbreviation: RT, room temperature.

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

Table 34. Laboratory Diagnosis of Cystitis and Pyelonephritis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Gram-negative bacteria			
Enterobacteriaceae:includes *Escherichia coli*, *Klebsiella* spp, *Proteus* spp, others *Pseudomonas* spp, other nonfermenting gram-negative rods	Routine aerobic culture Gram stain (optional, low sensitivity)	Midstream, clean-catch, or straight-catch urine	Closed sterile leak-proof container; refrigerate (4°C) or use urine transport tube unless delivery to lab ≤1 h is certain
Gram-positive bacteria			
Enterococcus spp *Staphylococcus aureus* *Staphylococcus saprophyticus* *Corynebacterium urealyticum* *Streptococcus agalactiae* (group B streptococci)	Routine aerobic culture Gram stain (optional, low sensitivity)	Midstream, clean-catch, or straight-catch urine	Closed sterile leakproof container; refrigerate (4°C) or use urine transport tube unless delivery to lab ≤1 h is certain
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	First-void urine	Prefer >20 mL urine, refrigerate (4°C) during transport
Virus			
Adenovirus	NAAT[a]	Midstream or clean-catch urine	Closed sterile container to lab within 1 h
BK polyoma virus	Quantitative NAAT[a] from urine, plasma, or serum	Blood Serum	EDTA or citrate blood collection tube, RT Clot tube, RT

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; RT, room temperature.

[a]No US Food and Drug Administration–cleared NAAT tests are available.

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 <10 000 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 >50 000 CFU/mL of a single organism obtained by either a suprapubic aspirate or catheterization is considered diagnostic [188]. More recent evidence would suggest that $\geq 10^4$ 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

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

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.

XI. GENITAL INFECTIONS

Both point-of-care and laboratory tests to identify the microbiological etiology of genital infections are described below. In addition, because recommendations exist for screening of genital infections for specific risk groups, these are also presented. In this section infections are categorized as follows: cutaneous

Table 35. Laboratory Diagnosis of Prostatitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Acute bacterial prostatitis			
Escherichia coli, other enteric bacteria *Pseudomonas* spp *Staphylococcus aureus* *Enterococcus* Group B streptococci	Aerobic culture	Midstream urine with or without expressed prostate secretions	Closed sterile container to laboratory within 1 h or refrigerate (4°C) if delayed transport
Chronic bacterial prostatitis			
Pathogens similar to acute bacterial disease	Gram stain or cell counts Aerobic culture	Midstream urine and expressed prostate secretions, seminal fluid	Closed sterile container to laboratory within 1 h or refrigerate (4°C) if delayed transport
Fungus			
Blastomyces dermatitidis *Coccidioides immitis* *Histoplasma capsulatum*	Fungal culture	Expressed prostate secretions, prostate biopsy	Closed sterile container to laboratory within 1 h or refrigerate (4°C) if delayed transport
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	First-void urine, expressed prostate secretions, prostate biopsy	Prefer >20 mL urine, refrigerate (4°C) during transport

genital lesions, vaginitis and vaginosis, urethritis and cervicitis, and infections of the female pelvis, including endometritis and pelvic inflammatory disease (PID). Testing in special populations, such as pregnant patients, children, and men who have sex with men (MSM) are noted where applicable but readers are referred to the more comprehensive guidelines referenced.

There is considerable overlap in symptoms and signs for many genital infections, and clinical diagnosis alone is neither sensitive nor specific. Thus, diagnostic testing is recommended for the following reasons: appropriate treatment can be focused for eradication, reduction of transmission as well as symptomatic relief, specific diagnosis has the benefit of increasing therapeutic compliance by the patient and the patient is more likely to comply with partner notification [195, 196].

Providers should also recognize that despite diagnostic testing, 25%–40% of the causes of genital infections or symptoms may not be specifically identified, and that many infections are acquired from an asymptomatic partner unaware of their infection. In fact, patients who seem to "fail" therapy and continue to exhibit symptoms and/or have positive tests for sexually transmitted infections (STIs) are most likely to have been reinfected by their sexual partner [197, 198]. Thus, referral for partners for specific testing and/or directed treatment is essential to prevent reinfection and is especially true for patients who may be pregnant or are HIV positive. Finally, because the vast majority of genital infections are STIs and communicable, they are a public health concern. Patients and their providers should note that positive tests for CT, GC, syphilis, chancroid, and HIV require

Table 36. Laboratory Diagnosis of Epididymitis and Orchitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacteria			
Chlamydia trachomatis *Neisseria gonorrhoeae*	NAAT Culture	Urethral swab or first void urine for NAAT Urine not suitable for culture	Specific collection system for each NAAT
Enteric bacteria *Staphylococcus aureus*	Aerobic culture and susceptibility test	Tissue aspirate or biopsy	Closed sterile container, refrigerate (4°C) if delay.
Virus			
Mumps Coxsackie Rubella EBV VZV	Serology Culture where available	Acute and convalescent serum Tissue aspirate or biopsy	Clot tube, RT Closed sterile container, refrigerate (4°C) if delay
Fungus			
Blastomyces dermatidis *Coccidioides immitis* *Histoplasma capsulatum*	Fungal culture	Tissue aspirate of biopsy	Closed sterile container, refrigerate (4°C) if delay
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	Tissue aspirate or biopsy	Closed sterile container, refrigerate (4°C) if delay

Abbreviations: EBV, Epstein-Barr virus; NAAT, nucleic acid amplification test; RT, room temperature; VZV, varicella zoster virus.

reporting in accordance with state and local statutory requirements by the laboratory and/or the provider. Reporting of additional STIs varies by state [195].

Key points for the laboratory diagnosis of genital infections:

- For vaginosis (altered vaginal flora) a Gram stain and recently available microbiome-based assays are more specific than culture and probe testing for *Gardnerella vaginalis* alone.
- Resistant *Candida* spp occur in 10%–15% of patients with recurrent yeast vulvovaginitis.
- Most laboratories use a reverse syphilis screening algorithm; treponemal specific test first (EIA/chemiluminescence immunoassay) followed by nontreponemal (rapid plasma reagin [RPR]) to confirm.
- Testing simultaneously for CT, GC, and *Trichomonas* is optimal for detection of the most common treatable STIs in female patients.
- High-risk individuals (eg, MSM) should have extragenital sites evaluated (rectal, oropharyngeal) for GC and CT.
- Screen for group B streptococcus at 35–37 weeks of pregnancy using both vaginal and rectal swabs; susceptibility of group B *Streptococcus* is not routinely performed unless the patient is penicillin allergic.
- Screen for HIV in each new pregnancy during the first prenatal visit or trimester as well as third trimester even in previously tested pregnancies and in sexually active patients aged 13–64 seeking evaluation for STIs.
- Undertake partner testing and/or treatment of positive index cases to prevent reinfection.
- Co-testing with hrHPV and cytology increases detection of cervical cancer compared to cytology alone.
- High-risk HPV and genotyping helps triage management of women >30 years of age.
- Recognized emerging diagnostic issues:
  - o *Mycoplasma genitalium* as a cause of nongonococcal urethritis in males and cervicitis and PID in females.
  - o Acquisition of hepatitis C virus by sexual transmission in MSM [195].
  - o Resistance of *N. gonorrhoeae* to antimicrobials [195].

A. Genital Lesions

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

Table 37. Laboratory Diagnosis of Genital Lesions

Etiologic Agents	Diagnostic Procedures	Optimum Specimen	Transport Issues and Optimal Transport Time
HSV-1 and HSV-2	NAAT[b]	Scraping or aspirate	Assay-specific; consult laboratory
In children with genital lesions, consider atypical VZV[a]	DFA	Scraping of lesion base rolled directly onto slide[a]	RT
	Culture (rarely performed)	Scraping of lesion base and placed in VTM//UTM[c]	RT, If >2 h, refrigerated
	Serology[d]	Serum	Clot tube, RT
HPV-16/18 genotyping Refer to guidelines for specific age, cytology, and triaging recommendations	DNA hybridization probe or NAAT for high-risk HPV types only[e]	Endocervical brush into liquid cytology medium or transport tube	RT, 48 h
Genital warts[f]	Histopathology; high-risk HPV testing not done on warts	Biopsy or scraping	Formalin container, RT, 2–24 h
Syphilis	Darkfield microscopy[g] Test is not widely available and specimen must be transported to laboratory immediately to visualize motile spirochetes	Cleanse lesion with gauze and sterile saline Swab of lesion base directly to slide	RT, immediately to laboratory
	DFA–*Treponema pallidum*[h,i]	Cleanse lesion with gauze and saline Swab of lesion base directly to slide. Clarify source, genital, oral, rectal	Slide should be dry before placing in holder and/or transporting to laboratory
	Serology Nontreponemal (VDRL or RPR)[j]	Serum	Clot tube, RT, 2 h
	Treponemal serology EIA/CIA or TPPA, FTA-ABS)[k,l]	Serum	Clot tube, RT, 2 h
Chancroid (*Haemophilus ducreyi*)[m]	Gram stain and culture[n]	Swab of lesion base without surface genital skin	RT immediately to laboratory
	NAAT[b]		
Lymphogranuloma venereum[m] (*Chlamydia* serovars L1, L2, L2a, L2b, L3)	Cell culture[o]	Swab of ulcer base, bubo drainage, rectum	RT, immediately to laboratory
	Serology MIF[p]	Serum	RT, 2 h
	Serology Complement fixation[q]	Serum	RT, 2 h
	NAAT[r]	Swab of ulcer base, bubo drainage, rectum	RT, 2 days; or refrigerate
Granuloma inguinale[m] (donovanosis) *Klebsiella granulomatis*	Giemsa or Wright stain in pathology. Visualization of blue rods with prominent polar granules	Scraping of lesion base into formalin	RT, 2 h
Scabies	Microscopic visualization	Collect parasite from skin scrapings using sterile scalpel blade with a drop of mineral oil to facilitate adherence of the organism[s]	RT, within 1 h
Lice	Macroscopic visualization	Hair should be submitted in a clean container	RT, 1 h

Abbreviations: CIA, chemiluminescence immunoassay; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody–absorbed; HPV, human papillomavirus; HSV, herpes simplex virus; MIF, microimmunofluorescent stain; NAAT, nucleic acid amplification test; RPR, rapid plasma reagin; RT, room temperature; TPPA, *Treponema pallidum* particle agglutination assay; UTM, universal transport medium; VDRL, Venereal Disease Research Laboratory; VTM, viral transport medium; VZV, varicella zoster virus.

[a]Epithelial cells are required for adequate examination and used to assess quality of the specimen collection; consider atypical VZV in children with genital lesions using DFA. Typical 3-well slide allows distinction between HSV-1, HSV-2, and VZV.

[b]Several NAATs are US Food and Drug Administration (FDA)–cleared. Specimen source and test availability are laboratory specific. Provider needs to check with laboratory for allowable specimen source and turnaround time. More sensitive than culture and DFA, especially when lesions are past vesicular stage.

[c]Check with laboratory; most are maintained and shipped at RT, ice not required.

[d]Serology can be non-specific for HSV-1 and HSV-2 differentiation; should be limited to patients with clinical presentation consistent with HSV but with negative cultures by NAAT; request type-specific glycoprotein G–based assays that differentiate HSV-1 and HSV-2.

[e]High-risk HPV testing currently recommended in women ≥30 years of age. HPV testing is not recommended for the diagnosis of HPV in a sexual partner or in patients aged ≤21 years (adolescents); options for women aged 21–29 years vary depending on cytology, HPV-16/18 genotyping in cytology negative and high-risk HPV positivity helps with triage.

[f]The diagnosis of genital warts is most commonly made by visual inspection; high-risk HPV testing is not recommended.

[g]Darkfield microscopy not widely available.

[h]Limited availability typically performed in public health laboratories.

[i]Viable organisms are not required for optimal test performance; clarify source (genital, oral, rectal) because nonpathogenic treponemes can be detected in nongenital sites.

[j]Nontreponemal tests (RPR and VDRL) are less sensitive in early and late disease, and become negative after treatment; do not use to test pregnant patients due to potential for false-positive results.

[k]Treponemal tests: EIA or CIA formats, TPPA, and FTA-ABS; monitor titers using same type of test and/or same laboratory; positive for life; human immunodeficiency virus (HIV)–infected patients may have unusual serologic responses.

[l]Treponemal test may be performed first with subsequent testing done with nontreponemal tests such as RPR (reverse testing algorithm). Confirmation with a second treponemal test different than the first is required in positive EIA/CIA but negative RPR tests. For laboratories that routinely perform the reverse algorithm, a special request for testing by RPR may be required when following positive syphilis patients for treatment effectiveness.

[m]Uncommon genital ulcers in the United States are typically diagnosed by clinical presentation, risk factors, and exclusion of syphilis and HSV; HIV testing should be part of workup.

[n]Gram stain with chancroid organisms shows small rods or chains in parallel rows, "school of fish"; culture requires special media and sensitivity is only 30%–70%. Testing should only be performed by laboratory that regularly performs this testing.

[o]Cell culture sensitivity about 30%; rectal ulcers in men who have sex with men.

[p]MIF titers ≥256 with appropriate clinical presentation suggests lymphogranuloma venereum (LGV).

[q]Complement fixation titers ≥64 with appropriate clinical presentation suggests LGV, sensitivity 80% at 2 weeks.

[r]NAATs for *C. trachomatis* (CT) will detect L1–L3 but do not distinguish these from the other CT serovars; typical lesion sites not FDA-cleared; some laboratories have validated rectal swabs; NAAT performed through the Centers for Disease Control and Prevention in outbreak situations [210].

[s]Place a drop of mineral oil on a sterile scalpel blade. Allow some of the oil to flow onto the papule. Scrape vigorously 6 or 7 times to remove the top of the papule. (Tiny flecks of blood should be seen in the oil.) Use the flat side of the scalpel to add pressure to the side of the papule to push the mite out of the burrow. Transfer the oil and scrapings onto a glass slide (an applicator stick can be used). Do not use a swab, which will absorb the material and not release it onto the slide. For best results, scrape 20 papules.

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:

- Cytology (Papanicolaou [Pap] smear) screening is only acceptable in women <30 years of age.
- High-risk HPV testing is unacceptable for routine management of women aged 21–29 years.
- Co-testing with cytology and hrHPV is preferred in women ≥30–65 years of age.
- High-risk HPV–negative and atypical squamous cells of undetermined significance (ASC-US) results should be followed with co-testing at 3 years, not 5.
- In general, results of cytology negative but hrHPV positive warrant HPV-16/18 genotype to identify patients at higher risk for progression to cervical cancer.

Prior management guidelines from the 2006 consensus guidelines for the management of women with abnormal cervical screening tests [203] retained include:

- Women <21 years of age do not need routine cervical screening.
- Endocervical specimens in liquid cytology medium have a higher sensitivity for detecting significant lesions and facilitate subsequent HPV testing in patients because tests can be done from the same specimen.
- High-risk HPV testing is not recommended for ages 21–29 as a routine test but is recommended for the purposes of triaging women >25 years of age with ASC-US or ASC-H (atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion).
- Only testing for hrHPV types associated with cervical cancer is appropriate.
- HPV-negative and ASC-US results are insufficient to allow exit from screening at age 65 years.
- In women >30 years of age, co-testing strategies with negative cytology but positive hrHPV reflex to 16/18 genotyping should be considered

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

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

Table 38. Laboratory Diagnosis of Bacterial Vaginosis, Yeast Vaginitis[a], and Trichomoniasis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Yeast (pH <4.5[b])	Saline wet mount and 10% KOH[c]	Swab of vaginal discharge	Submitted in 0.5 mL saline or transport swab[d], RT, 2 h
	Culture[e]	Swab of vaginal discharge	Submitted in transport swab, RT, 12 h
	DNA hybridization probe[f]	Swab of vaginal discharge[f]	Lab provided transport RT, 7 d, or manufacturer's recommendations
Bacterial vaginosis (pH >4.5[b])	Wet mount and 10% KOH[g]	Swab of vaginal discharge	Submitted in 0.5 mL saline or transport swab, RT, 2 h
	Quantitative Gram stain[h]	Swab of vaginal discharge	Place directly into transport swab tube, RT, 12 h
	DNA hybridization probe[f]	Swab of vaginal discharge[f]	Lab provided transport, RT, 7 d or manufacturer's recommendations
Trichomoniasis (pH >4.5)[b]	[i]	Vaginal, endocervical swab, urine or liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	Lab provided transport, RT, 7 d (or manufacturer's recommendation)
	Rapid antigen test[j]	Swab of vaginal epithelium/discharge	Submitted in transport swab or saline, RT, 24 h
	DNA hybridization probe[f]	Swab of vaginal discharge[f]	RT, 7 d
	Culture[k]	Swab of vaginal discharge	Place directly into InPouch TV Culture system, RT, 48 h
	Saline wet mount[l]	Swab of vaginal discharge	Submitted in saline, RT, 30 min (optimal) – 2 h

Abbreviations: KOH, potassium hydroxide; RT, room temperature; TV, *Trichomonas vaginalis*.

[a]Multiplex nucleic acid amplification test (NAAT) Max Vaginal panel (Becton Dickinson, Sparks, Maryland). Multiplex NAAT for *Candida albicans* and resistant species (*Candida glabrata/krusei*), microbiome-based bacterial vaginosis, and TV. US Food and Drug Administration (FDA)–cleared for use in symptomatic females.

[b]pH of vaginal discharge for each condition listed when using pH strips as a point-of-care test.

[c]Sensitivity of wet mount between 40% and 80%.

[d]Culturette (BD Microbiology Systems, Sparks, Maryland), Eswab (Copan Diagnostics, Murietta, California), or similar product.

[e]Consider culture in recurrent cases and when wet mount/KOH is negative.

[f]Affirm VP III Assay (Becton Dickinson); does not rely on viable organisms for optimal test performance; special transport tube required; detects *Gardnerella vaginalis* as an organism associated with BV, yeast vaginitis (*C. albicans* only), and TV. FDA-cleared for vaginal specimens from symptomatic female patients only. *Trichomonas* sensitivity not as good as NAAT.

[g]Amine or fishy odor, "whiff test" positive when KOH added, lack of white blood cells, and presence of clue cells.

[h]Quantitative Gram stain most specific procedure for bacterial vaginosis; culture not recommended; testing and treatment recommended in symptomatic pregnant patients to reduce postpartum endometritis.

[i]Many assays are currently FDA-cleared. Specific use (screening as well as diagnostic, female and/or male); specific sources and self-collection vary depending on test. Same specimen and collection device often is used for *Chlamydia trachomatis*/*Neisseria gonorrhoeae* NAAT. Provider needs to check with laboratory for availability.

[j]OSOM Trichomonas Rapid Test (Genzyme, Diagnostics, Cambridge, Massachusetts); does not require live organisms for optimal test performance, sensitivity ranges from 62% to 95% compared to culture and NAAT in symptomatic and asymptomatic patients, with best results in symptomatic patients.

[k]InPouch TV culture system (Biomed Diagnostics, White City, Oregon) allows both immediate smear review by wet mount and subsequent culture; not widely available, sensitivity approximately 70% compared to NAAT methods.

[l]Wet mount for trichomonads requires live organisms to visualize movement and has poor sensitivity.

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].

C. Urethritis/Cervicitis

Urethritis and cervicitis share common signs and symptoms and infectious etiologies in male and female patients, respectively. Table 39 combines the diagnostic tests used to identify the pathogens common to both. In addition, because screening for CT and GC has reduced the repercussions related to infections and subsequent PID, the following guidelines for screening women for CT and GC have been presented by the US Preventive Services Task Force in 2014 (available at: https://www.uspreventiveservicestaskforce.org/Page/Document/RecommendationStatementFinal/chlamydia-and-gonorrhea-screening).

Annual CT Screening

- Sexually active women aged ≤25 years and those pregnant
- Older women with the following identified risk factors:
  - new sex partner
  - multiple partners
  - partner with an STI
  - inconsistent condom use, not in a monogamous relationship
  - previous or coexisting STI
  - exchanging sex for money or drugs

GC Screening (Consider Local Epidemiology and Risk)

- Sexually active women aged ≤25 years and those pregnant
- Similar criteria as above for CT

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

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

Table 39. Laboratory Diagnosis of Pathogens Associated With Cervicitis/Urethritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Chlamydia trachomatis	NAAT[a]	Urine Endocervical, vaginal, and/or urethral swab (rectum, pharynx, conjunctiva, liquid-based cytology)[b,c]	Laboratory-provided transport device, RT, 2 d
	Culture[d]	Endocervical, urethral, conjunctival, NP, pharynx, or rectal swab	Laboratory-provided transport device, refrigerate (4°C); <2 h
	DFA test[e]	Conjunctival swab	Transport medium, RT, 2 h
Neisseria gonorrhoeae	Gram stain[f]	Urethral discharge	Smear on slide directly or submit swab in transport medium, RT, immediately
	NAAT[a]	Urine Endocervical, vaginal, and/or urethral swab (Rectal, pharynx, conjunctiva, liquid-based cytology specimen)[b,c]	Laboratory-provided transport device, RT, 2 d
	Culture[g]	Endocervical, urethral, conjunctival, nasopharyngeal, pharynx, rectal swab	Transport medium, RT, ≤1 h Do not refrigerate specimen
Trichomonas vaginalis	NAAT[c]	Vaginal, endocervical swab, urine and liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	Laboratory-provided transport device, RT, 2 d
	Rapid antigen test[h]	Endocervical swab	Laboratory-provided transport device, RT, 24 h
	DNA hybridization probe[i,j]	Endocervical or vaginal swab	Laboratory-provided transport device, RT, 7 d
	Culture[k]	Endocervical or urethral swab	Direct inoculation into InPouch TV culture system, 2–5 d
	Saline wet mount[l]	Endocervical or urethral swab	Submit in 0.5 mL saline, 30 min–2 h
Herpes simplex virus	DFA[m]	Scraping of lesion base	Apply to slide at bedside, RT, 24 h
	Culture	Scraping of lesion base	Place in VTM/UTM RT
	NAAT[n]	Scraping of lesion or swab of discharge	Laboratory-provided transport device, assay-specific; consult laboratory

Abbreviations: DFA, direct fluorescent antibody; NAAT, nucleic acid amplification test; NP, nasopharyngeal; RT, room temperature; UTM, universal transport medium; VTM, viral transport medium.

[a]Current US Food and Drug Administration (FDA)–cleared NAATs for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and *Trichomonas vaginalis* (TV) refer to: https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm.

[b]Pharynx and rectal specimens in men who have sex with men (requires laboratory validation for those specimen types).

[c]Some tests are FDA-cleared for both screening as well as diagnosis of TV in women. Some only for symptomatic patients. Multiple specimen types and transport can often be used for multiple sexually transmitted infections such as GC, CT, and TV. Testing for males and alternate sites has been validated by some laboratories. Provider needs to check with laboratory for availability.

[d]Not as sensitive as NAATs.

[e]Epithelial cells are required for adequate examination.

[f]Gram stain in males only; 10–15 white blood cells per high-powered field and intracellular gram-negative diplococci (gndc): 95% specific for GC; intracellular gndc seen, only 10%–29% specific for GC.

[g]Culture allows for antimicrobial susceptibility testing; culture sensitivity may be better when direct inoculation of specimen to selective media with carbon dioxide tablet at patient bedside; vancomycin in media may inhibit some GC strains.

[h]OSOM Trichomonas Rapid Test (Genzyme, Diagnostics, Cambridge, Massachusetts); does not require live organisms for optimal test performance, sensitivity ranges from 62% to 95% compared to culture and NAAT in symptomatic and asymptomatic patients, with best results in symptomatic patients.

[i]Check for availability. A reference laboratory may be needed.

[j]Affirm VP III Assay (Becton Dickinson, Sparks, Maryland); does not rely on viable organisms for optimal test performance; special transport tube required; detects *Gardnerella vaginalis* as an organism associated with bacterial vaginosis, yeast vaginitis (*Candida albicans* only), and TV. FDA-cleared for vaginal specimens and symptomatic female patients only. *Trichomonas* sensitivity not as good as NAATs.

[k]InPouch TV culture system (Biomed Diagnostics, White City, Oregon) allows both immediate smear review by wet mount and subsequent culture; not widely available. Sensitivity approximately 70% compared to NAAT methods.

[l]Wet mount for trichomonads requires live organisms to visualize movement; sensitivity 60%.

[m]Not widely available; reference test for some specimens; sensitivity approximately 70% compared to NAATs.

[n]Currently many FDA-cleared. Check with laboratory on available sources validated and potential sex and age restrictions.

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

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].

XII. SKIN AND SOFT TISSUE INFECTIONS

Cutaneous infections, often referred to as skin and soft tissue infections (SSTIs), occur when the skin's protective mechanisms fail, especially following trauma, inflammation, maceration from excessive moisture, poor blood perfusion, or other factors that disrupt the stratum corneum. Thus, any compromise of skin and skin structure provides a point of entry for a myriad of exogenous and endogenous microbial flora that can produce a variety of infections. Infections of the skin and soft tissue are often classified as primary pyodermas, infections associated with underlying conditions of the skin, and necrotizing infections. Representative primary cutaneous infections of the skin include cellulitis, ecthyma, impetigo, folliculitis,

Table 40. Laboratory Diagnosis for Pathogens Associated With Pelvic Inflammatory Disease and Endometritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Mixed anaerobic organisms Vaginal flora Enterobacteriaceae, enterococci, group A and B streptococci *Mycoplasma* *Actinomyces* spp[a]	Blood cultures and antimicrobial susceptibilities to assess unusual causes of PID or endometritis	Blood, 2 separate 20-mL venipuncture collections	Inject into blood culture bottles at bedside, RT, 1 h
	Gram stain[b] Aerobic and anaerobic culture[c]	Endometrium, tubo-ovarian abscess and/or fallopian tube contents	Place in or inject into sterile anaerobic container[d], RT, 30 min
	Histology for evidence of endometritis	Endometrial biopsy	Sterile container, RT, 30 min Formalin container, RT, 30 min–4 h
Neisseria gonorrhoeae[e] *Chlamydia trachomatis* *Trichomonas vaginalis* *Mycoplasma genitalium*	NAAT	Urine, endocervical swab	Laboratory-provided transport device, RT, 2 d
HIV	Serologic testing	Serum, plasma	Clot tube, RT, 2 h

Abbreviations: EIA, enzyme immunoassay; HIV, human immunodeficiency virus; NAAT, nucleic acid amplification test; PID, pelvic inflammatory disease; RT, room temperature.

[a]*Actinomyces* spp are an uncommon cause of PID.

[b]Gram stain may aid in identification of significant pathogen.

[c]Limited identification and antimicrobial susceptibility testing when cultures show multiple mixed aerobic and anaerobic organisms.

[d]Invasive specimens obtained by laparoscopic or other sterile technique.

[e]In patients with late-appearing postpartum endometritis, consider chronic and/or asymptomatic sexually transmitted infections such as chlamydia.

furunculosis, and erysipelas and are commonly caused by a narrow spectrum of pyogenic bacteria (*Staphylococcus aureus* and/or *Streptococcus pyogenes* [group A *Streptococcus*]). Secondary infections are often extensions of preexisting lesions (traumatic or surgical wounds, ulcers), which serve as the primary portal of entry for microbial pathogens and are often polymicrobial (mixed aerobic and anaerobic microorganisms) involving subcutaneous tissue. Diabetic foot infections (DFIs) typically originate in a wound, secondary to a neuropathic ulceration. Anaerobic bacteria are important and predominant pathogens in DFIs and should always be considered in choosing therapeutic options. The majority of DFIs are polymicrobial but gram-positive cocci, specifically staphylococci, are the most common infectious agents. *Pseudomonas aeruginosa* is involved in the majority of chronic DFIs but its relevance related to treatment decisions is not clear. Surface cultures of such wounds, including decubitus ulcers, are not valuable, as they usually represent colonizing microbes, which cannot be differentiated from the underlying etiologic agent. Tissue biopsies after thorough debridement, or bone biopsies through a debrided site, are most valuable. Necrotizing cutaneous infections, such as necrotizing fasciitis, are usually caused by streptococci (and less often by MRSA or *Klebsiella* spp), but can also be polymicrobial. The infection usually occurs following a penetrating wound to the extremities, is often life-threatening, and requires immediate recognition and intervention. On rare occasions, necrotizing fasciitis occurs in the absence of identifiable trauma.

For the common forms of SSTIs, cultures are not indicated for uncomplicated infections (cellulitis, subcutaneous abscesses) treated in the outpatient setting. Whether cultures are beneficial in managing cellulitis in the hospitalized patient is uncertain and the sensitivity of blood cultures in this setting is low. Cultures are indicated for the patient who requires operative incision and drainage because of risk for deep structure and underlying tissue involvement and cases of therapeutic failure [223].

In this section, cutaneous infections, involving skin and soft tissue, have been expanded and categorized as follows: trauma associated, surgical site, burn wounds, fungal, human and animal bites, and device related. Although the majority of these infections are commonly caused by *S. aureus* and *S. pyogenes*, other microorganisms, including fungi and viruses, are important and require appropriate medical and therapeutic management. It is important that the clinician be familiar with the extent or limitation of services provided by the supporting laboratory. For example, not all laboratories provide quantitative cultures for the assessment of wounds, especially burn wounds. If a desired service or procedure is not available in the local microbiology laboratory, consult with the laboratory so that arrangements can be made to transfer the specimen to a qualified reference laboratory with the understanding that turnaround times are likely to be longer, thus extending the time to receipt of results.

A major factor in acquiring clinically relevant culture and associated diagnostic testing results is the acquisition of appropriate specimens that represent the group of diseases discussed in this section. Guidelines for obtaining representative specimens are summarized as follows:

Key points for the laboratory diagnosis of SSTIs:

- Do not use the label "wound" alone. Be specific about body site and type of wound (for example "human bite wound, knuckle").
- The specimen of choice is a biopsied sample of the advancing margin of the lesion. Pus alone or a cursory surface swab is inadequate and does not represent the disease process.
- Do not ask the laboratory to report everything that grows.

A. Burn Wound 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

Table 41. Laboratory Diagnosis of Burn Wound Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Staphylococcus aureus Coagulase-negative staphylococci *Enterococcus* spp *Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae* *Serratia marcescens* *Proteus* spp *Aeromonas hydrophilaa* *Bacteroides* spp and other anaerobes	Aerobic, quantitative culture/AST	Blood culture Surface swab Tissue (punch biopsy)	RT, <12 h, aerobic RT, <2 h, transport medium No formalin, keep moist
	Histopathology	Tissue (punch biopsy)	Submit in formalin, RT, 2 h
	Anaerobic culture	Tissue biopsy or aspirate (swab may not represent the disease process)	Anaerobic transport tubes, prereduced media; RT, <2 h
	NAAT for MRSA and *S. aureus* only	Swab from manufacturer[b]	Laboratory-provided transport device, RT, <2 h
Fungi			
Candida spp *Aspergillus* spp *Fusarium* spp *Alternaria* spp Zygomycetes	Fungal culture	Tissue biopsy	RT, <30 min, no formalin, keep moist
	Fungal blood culture	Blood; 2-4 cultures per 24-h period	Lysis-centrifugation tube or broth-based blood culture bottles, RT, <2 h
Viruses			
Herpes simplex virus Cytomegalovirus Varicella zoster virus	Tissue culture NAAT, where applicable and laboratory-validated	Tissue (biopsy/aspirate)	Viral transport medium or laboratory-provided transport device

Abbreviations: AST, antimicrobial susceptibility test; MRSA, methicillin-resistant *Staphylococcus aureus*; NAAT, nucleic acid amplification test; RT, room temperature.

[a]Electrical burns; potential for transmission from leeches.

[b]Xpert MRSA/*S. aureus* skin and soft tissue infection (Cepheid, Sunnyvale, California).

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

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

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.

Table 42. Laboratory Diagnosis of Human Bite Wound Infections

Etiologic Agents	Diagnostic Procedures[a]	Optimum Specimens	Transport Issues and Optimal Transport Time
Bacterial			
Aerobes Mixed aerobic and anaerobic oral flora	Aerobic/anaerobic culture Gram stain	Tissue Biopsy/aspirate	Anaerobic transport conditions/vials

[a]There is no utility in collecting a specimen at the time of the bite; collect samples only if infection occurs.

D. Trauma-Associated Cutaneous 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

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

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,

Table 43. Laboratory Diagnosis of Animal Bite Wound Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Bacterial[a]			
Actinobacillus spp *Capnocytophaga* spp *Erysipelothrix rhusiopathiae*	Aerobic/anaerobic culture Gram stain	Tissue/biopsy/aspirate	Anaerobic transport container[b] Be certain to provide sufficient volume of sample for complete culture and Gram stain evaluation; RT, <2 h
Pasteurella spp *Streptobacillus* spp	Blood culture	Blood; 2-4 cultures per 24 h	Blood culture bottles, RT, <2 h
Mycobacterium fortuitum *Mycobacterium kansasii*	Aerobic culture Acid-fast culture Acid-fast stain	Tissue/biopsy/aspirate	Sterile container RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Transport in formalin, RT, 2 h–24 h

Abbreviation: RT, room temperature.

[a]Additional potential pathogens to consider: *Staphylococcus intermedius*, *Bergeyella zoohelcum*, *Propionibacterium* spp, *Filifactor* spp, *Moraxella* spp, *Neisseria* spp, *Kingella* spp, *Pseudomonas fluorescens*, *Halomonas venusta*, Centers for Disease Control and Prevention (CDC) group EF-4, CDC NO-1, *Peptococcus* spp, rabies, herpes B, or other viruses (refer to Section XIV).

[b]Anaerobic transport media preserve all other organisms for culture.

Table 44. Laboratory Diagnosis of Trauma-Associated Cutaneous Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Bacterial			
Staphylococcus aureus Group A, B, C, and G streptococci *Aeromonas hydrophila* and other *Aeromonas* spp *Vibrio vulnificus* *Bacillus anthracis*[b] *Clostridium tetani*[c] *Corynebacterium* spp Mixed aerobic/anaerobic flora (cutaneous origin)	Aerobic/anaerobic culture NAAT[a]	Surgical tissue Biopsy/aspirate	Aerobic/anaerobic conditions or anaerobic transport device; keep tissue moist
	Blood culture	Blood	Aerobic/anaerobic blood culture bottles, RT, <2 h
	Histopathology	Surgical tissue Biopsy/aspirate	Formalin container, RT, 2 h–24 h
Mycobacterium spp *Nocardia* spp	Mycobacterial culture Acid-fast smear	Tissue/biopsy/aspirate	Sterile container, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2 h–24 h
Fungal			
Aspergillus spp *Sporothrix schenckii* *Histoplasma capsulatum* *Blastomyces dermatitidis* *Coccidioides immitis* *Penicillium marneffei* Yeasts (*Candida/Cryptococcus* spp) Other filamentous fungi Zygomycetes Dematiaceous molds	Fungal culture Calcofluor-KOH preparation	Surgical tissue Biopsy/aspirate	Aerobic transport device Keep tissue moist; avoid formalin fixation
	Histopathology	Surgical tissue Biopsy/aspirate	Formalin container, RT, 2 h–24 h

Abbreviations: KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

[a]There is an US Food and Drug Administration –cleared NAAT for direct detection of *S. aureus* and methicillin-resistant *S. aureus* from swabs of wounds and pus.

[b]Potential bioterrorism agent: if suspicious, notify laboratory in the interest of safety.

[c]*Clostridium tetani* can also be an etiological agent of trauma-associated infections in rare cases. This is usually a clinical diagnosis rather than a laboratory diagnosis.

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

Table 45. Laboratory Diagnosis of Surgical Site Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Bacterial			
Staphylococcus aureus Coagulase-negative staphylococci β-hemolytic streptococci (groups A, B, C, and G) Nonhemolytic streptococci Enterococci *Acinetobacter* spp *Pseudomonas aeruginosa* Enterobacteriaceae Indigenous/exogenous aerobic/anaerobic flora	Gram stain Aerobic culture and AST NAAT[a]	Tissue/biopsy/aspirate	Keep tissue moist; aerobic transport, RT, <2 h
	Anaerobic culture (if appropriate)	Tissue/biopsy/aspirate	Anaerobic transport device RT, <2 h
	Blood culture	Aerobic and anaerobic bottles	RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h RT, indefinite
Mycoplasma hominis and *Legionella pneumophila* (rare but possible agents in specific situations)[b]	Culture (mycoplasma culture requires special handling)	Tissue/biopsy/aspirate	Special transport medium; check with laboratory if available
Mycobacterium spp–rapid growers	Acid-fast stain and culture	Tissue/biopsy/aspirate	Aerobic transport device Sterile container RT, <2 h
Fungi			
Candida spp	Fungal culture Calcofluor-KOH preparation	Tissue/biopsy/aspirate	Aerobic transport device Sterile container RT, <2 h
	Fungal blood culture	Blood	Lysis-centrifugation blood culture tube or aerobic blood culture bottles, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h

Abbreviations: AST, antimicrobial susceptibility test; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

[a]There is an US Food and Drug Administration–cleared NAAT for direct detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* from swabs of wounds and pus.

[b]*Mycoplasma hominis* has caused infections post–joint surgery and post–abdominal surgery, particularly after cesarean deliveries. A series of sternal wound infections due to *Legionella* spp were traced to contamination of the hospital water supply. A post–hip surgery *Legionella* infection occurred after skin cleansing with tap water. Proper water treatment should remove the risk for such infections.

from the skin or gastrointestinal tract; for the remaining drain types, skin flora represent the predominate pathogens.

G. Cutaneous Fungal 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

Table 46. Laboratory Diagnosis of Fungal Infections of Skin and Subcutaneous Tissue

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Dermatophytes/tineas			
Epidermophyton spp *Trichophyton* spp *Microsporum* spp	Fungal culture Calcofluor-KOH preparation	Skin scrapings/hair follicles/nail scrapings	Sterile transport container Aerobic conditions RT, <4 h
	Histopathology	Tissue/biopsy	Formalin container, RT, 2–24 h
Dematiaceous (darkly pigmented) filamentous fungi			
Scedosporium/Pseudallescheria spp *Exophiala* spp *Cladosporium* spp *Phialophora* spp *Alternaria* spp *Bipolaris* spp	Fungal culture Calcofluor-KOH preparation	Tissue/biopsy/aspirate	Sterile transport container Aerobic conditions RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h
Dimorphic			
Histoplasma capsulatum *Blastomyces dermatitidis* *Coccidioides immitis* *Paracoccidioides brasiliensis* *Penicillium marneffei* *Sporothrix schenckii*	Fungal culture Urine antigen (*Histoplasma; Blastomyces)* Calcofluor-KOH preparation	Tissue/biopsy/aspirate Urine	Sterile transport container Aerobic conditions Sterile cup; RT <2 h
	Fungal serology	Serum	Clot tube, RT, <2 h
	Blood culture	Lysis-centrifugation vials or blood; 2 sets	RT, <2 h Aerobic blood culture bottles, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h
Yeast-like fungi			
Candida spp *Cryptococcus neoformans* *Trichosporon* spp *Geotrichum* spp *Malassezia* spp	Fungal culture Calcofluor-KOH preparation stain	Tissue/biopsy/aspirate Blood; 2 sets	Sterile transport container Aerobic conditions RT, <2 h Aerobic blood culture bottles, RT, <2 h
	Blood culture	Blood; 2 sets	Aerobic blood culture bottle or lysis/centrifugation blood culture, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h
Other fungi			
Aspergillus spp *Fusarium* spp Zygomycetes	Fungal culture Calcofluor-KOH preparation	Tissue/biopsy/aspirate Blood; 2 sets (*Fusarium* only)	Sterile transport container Aerobic conditions RT, <2 h Aerobic blood culture bottles or lysis/centrifugation blood cultures, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2–24 h

Abbreviations: KOH, potassium hydroxide; RT, room temperature.

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

Table 47. Laboratory Diagnosis of Tick-borne Infections

Etiologic Agents[a]	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Bacteria			
Relapsing fever borreliae *Borrelia hermsii* (western US) *Borrelia parkeri* (western US) *Borrelia turicatae* (southwestern US) *Borrelia mazzottii* (southern US)	Primary test[b]: Darkfield microscopy or Wright, Giemsa, or Diff-Quik stains of peripheral thin or/ and thick blood smears. Can be seen in direct wet preparation of blood in some cases.	Blood, bone marrow	EDTA or citrate blood tube, RT, ≤30 min
	Others tests NAAT Culture[c] Serologic testing[d]	Serum, blood, body fluids Blood, body fluids Serum	Clot tube for serum; sterile tube or citrate tube for body fluids, RT, within 2-4 h
Borrelia burgdorferi sensu lato complex (Lyme borreliosis)[e] *Borrelia burgdorferi* (US)[g] *Borrelia mayonii* (US) *Borrelia garinii* (Europe, Asia) *Borrelia afzelii* (Europe, Asia)	Early, localized Lyme disease with EM[f]	Testing not routinely recommended (See NAAT below)	
	Early, disseminated if EM or multiple EM rash absent (weeks through months after tick bite) or late (months through years after tick bite) in untreated patients: Primary test: 2-tier testing (acute- and convalescent-phase sera optimal) = EIA IgG and IgM antibody screening. If EIA result is positive or equivocal, confirm with IgG and IgM Western or immunoblots[h]. NOTE: A Western or immunoblot should not be performed unless an initial EIA is reported as positive or equivocal.	Serum	Clot tube, RT, ≤ 2 h
	Early Lyme neuroborreliosis: 2-tiered testing algorithm[i] Late Lyme neuroborreliosis CSF/serum antibody index	Serum Paired serum and CSF, collected within 24 h	Clot tube, RT, ≤ 2 h Clot tube for serum, sterile tube for CSF, RT, ≤1 h
	NAAT[j]	Biopsy specimens of infected skin, synovial fluid or tissue, etc	Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen at –70°C.
Borrelia miyamotoi (*B. miyamotoi* infection, hard tick-borne relapsing fever)	Primary test for acute infection: NAAT	Blood	Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen at –70°C
	Serology: EIA for detection of antibodies to recombinant GlpQ antigen	Serum	Clot tube, RT, <2 h
Anaplasma phagocytophilum (human granulocytotropic anaplasmosis)[k]	Primary test for acute infection: NAAT	Blood	EDTA anticoagulant tube Transport on ice; ≤1 h
	Alternative primary test (if experienced technologists available/NAAT unavailable): Wright or Giemsa stain of peripheral blood or buffy coat leukocytes during week first week of infection.	Blood	EDTA or citrate tube, RT, ≤1 h
	Serology: Acute and convalescent IFA titers for IgG-class antibodies to *A. phagocytophilum* antibodies[l]	Serum	Clot tube, RT, ≤2 h
	Immunohistochemical staining of *Anaplasma* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver, and lung)	Formalin container, RT, ≤2 h
Ehrlichia chaffeensis (human monocytotropic ehrlichiosis) *Ehrlichia muris* *Ehrlichia ewingii*[k,l]	Primary test for acute infection: NAAT NOTE: Only definitive diagnostic assay for *E. ewingii*)	Whole blood	Heparin or EDTA anticoagulant tube Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen.
	Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection	Blood	EDTA anticoagulant tube, RT, ≤1 h
	Serology: acute and convalescent IFA titers for *Ehrlichia* IgG-class antibodies[m]	Serum	Clot tube, RT, ≤2 h
	Immunohistochemical staining of *Ehrlichia* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)	Formalin container, RT, ≤2 h
Rickettsia rickettsii (RMSF)[n,o] Other spotted fever group *Rickettsia* spp (mild spotted fever) *R. typhi* (murine typhus) *R. akari* (rickettsialpox) *R. prowazekii* (epidemic typhus)	Serology: acute and convalescent IFA for *Rickettsia* sp IgM and IgG antibodies[m]	Serum	Clot tube, RT, ≤2 h
	NAAT	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)	Sterile container Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen.
	Immunohistochemical staining of spotted fever group rickettsiae antigens (up to first 24 h after antibiotic therapy initiated) in formalin-fixed, paraffin-embedded specimens	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)	Formalin container, RT, ≤2 h
Protozoa			
Babesia microti *Babesia* spp	Primary Test: Giemsa, Wright, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood Second choice: EDTA Vacutainer tube	For whole blood, prepare smears immediately RT, ≤30 min
	Primary test for acute infection: NAAT	Blood	EDTA anticoagulant tube, RT, ≤1 h
	Serology: acute and convalescent IFA titers for *Babesia* IgG-class antibodies[p] NOTE: Not recommended for acute infection.	Serum	Clot tube, RT, ≤2 h

Table 47. Continued

Etiologic Agents[a]	Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Times
Virus			
Colorado tick fever virus	Virus-specific IFA-stained blood smears	Blood	EDTA anticoagulant tube, RT, ≤2 h
	Serology: IFA titers or complement fixation[q]	Serum	Clot tube, RT, ≤2 h
Powassan/deer tick virus	Primary test: IgM capture EIA (available only through state departments of public health)	Serum	Clot tube, RT, ≤2 h
	NAAT	Blood, CSF, brain (biopsy or autopsy)	Frozen or in RNAlater/Trizol

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; EM, erythema migrans; IFA, immunofluorescent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RMSF, Rocky Mountain spotted fever; RT, room temperature; US, United States.

[a]Other tick-borne diseases should be considered if patients have traveled to international destinations. Because travel between North America and Europe is common, Lyme borreliosis caused by *Borrelia garinii* and *Borrelia afzelii* have been included in the table. Tick-borne rickettsial diseases such as African tick-bite fever (ATBF) or Mediterranean spotted fever (MSF) occur worldwide and might have epidemiologic, seasonal, and clinical features that differ from those observed in the United States [253]. Of note, tick-borne disease caused by *Rickettsia parkeri* is emerging; this organism has a similar clinical presentation as ATBF and MSF with fever, headache, eschars, and regional lymphadenopathy [255]. State laboratories and the Centers for Disease Control and Prevention can perform NAATs on swab or biopsy.

[b]Organisms are best detected in blood while a patient is febrile. With subsequent febrile episodes, the number of circulating spirochetes decreases. Even during initial episodes, organisms are seen only 70% of the time.

[c]Special media and technical expertise is required for culture of *Borrelia* spp that cause relapsing fever. A centrifugation-based enrichment method followed by Giemsa staining is a rapid and viable approach [256].

[d]Not valuable for an immediate diagnosis; however, serologic testing is available through public health and some private laboratories. An acute serum (obtained within 7 days of the onset of symptoms) and convalescent serum (obtained at least 21 days after the onset of symptoms) should be submitted for testing. Of significance, early antibiotic treatment can blunt the antibody response and antibody levels may fall quickly during the months after exposure.

[e]To date, 18 genomic species are reported in the literature; 3 are confirmed agents of localized, disseminated, and late manifestations of Lyme disease and are listed in the table. Another 9 species have been described with possible pathogenic potential [257]. Serologic assays used in North America are designed to detect antibodies to *B. burgdorferi* sensu stricto. These assays, particularly the blots, are insensitive for detection of *B. garinii*, *B. afzelii*, or *B. mayonii* antibodies. Immunoblots for detection of antibodies to *B. garinii* or *B. afzelii* are available at select commercial reference laboratories. Lyme VlsE-based ELISAs will detect infection with *B. garinii* or *B. afzelii*. Also, Lyme C6 testing is available and US Food and Drug Administration (FDA)–approved if suspicious for acquiring *B. garinii* or *B. afzelii*.

[f]EM is the only manifestation of Lyme disease in the United States that is sufficiently distinctive to allow clinical diagnosis in the absence of laboratory confirmation. Positive culture rates for secondary EM lesions, primary EM lesions, and large-volume (≥9 mL) blood or plasma specimens are 90%, 60%, and 48%, respectively [258]. If skin is biopsied, >1 biopsy sample should be taken for culture due to uneven distribution of spirochetes; disinfect the skin prior to collection and submit tissues in sterile saline. Culture is rarely performed outside of research settings. Serologic testing in patients with early localized Lyme disease is insensitive and associated with a low negative predictive value due to the low level of antibodies present at this stage of infection.

[g]*Ixodes* ticks have a broad host range, thereby increasing the chance of acquiring multiple pathogens from reservoir hosts. Thus, patients with one documented tick-transmitted disease are at increased risk for infection with another tick-transmitted organism. Patients with a diagnosis of Lyme disease have demonstrated immunoserologic evidence of coinfection with *Babesia microti*, *Anaplasma phagocytophilum*, or *Ehrlichia* spp; coinfection with tick-borne encephalitis virus (including Powassan/deer tick virus) should also be considered [259].

[h]Perform an IgM and an IgG Western blot (WB) during the first 4 weeks of illness on a patient with a positive EIA. An IgM WB is not interpretable after a patient has had symptoms for >1 month's duration because the likelihood of a false-positive test result for a current infection is high in these persons; therefore, in patients with symptoms >4 weeks, only test an IgG WB (http://www.cdc.gov/lyme/healthcare/clinician_twotier.html). In addition, a positive IgM WB is considered positive only if 2 of the following 3 bands are present: 24 kDa, 39 kDa, and 41 kDa. Similarly, a positive IgG WB is considered positive only if 5 of the following 10 bands are present: 18 kDa, 21 kDa, 28 kDa, 30 kDa, 39 kDa, 41 kDa, 45 kDa, 58 kDa, 66 kDa, and 93 kDa. Laboratories performing this testing are strongly encouraged to report only the presence/absence of these specified bands since misinterpretation of Lyme disease WBs can otherwise possibly occur.

[i]Laboratory assays for the diagnosis of neuroborreliosis are of limited clinical value [260].

[j]Other Lyme-associated diseases can be diagnosed by NAAT (turnaround time [TAT], 24-48 h) or culture (TAT, 3 days to 6–12 weeks). Acceptable specimens for multiple erythemata or borrelial lymphocytoma, Lyme carditis, Lyme arthritis, and acrodermatitis are skin biopsy, endomyocardial biopsy, synovial fluid or biopsy, and skin biopsy, respectively [259, 261]. Although *Borrelia* can be detected by NAAT in blood or CSF, its usefulness for the diagnosis of Lyme disease in these specimen sources is limited. For example, *Borrelia* DNA is detected in the blood of less than half of patients in the early acute stage of disease when the EM rash is present, and if symptoms of Lyme disease have been present for a month or more, spirochetes can no longer be found in blood. Similarly, NAAT testing of CSF specimens is positive in only about one-third of US patients with early neuroborreliosis, and is even less sensitive in patients with late neurologic disease. The utility of testing synovial fluid and other specimen types is not well established and should be considered only under special circumstances and skin biopsy is not generally recommended because patients with EM can be reasonably diagnosed and treated on the basis of history and clinical signs alone.

[k]Communication with the laboratory is of paramount importance when ehrlichiosis is suspected, to ensure that Wright-stained peripheral blood smears will be carefully examined for intra-cytoplasmic inclusions (morulae) in either monocytes or neutrophils or bands.

[l]Serologic testing for *Anaplasma phagocytophilum* is associated with a specificity of 83%–100%, with cross-reactivity occurring in patients infected with *Ehrlichia* spp, *Rickettsia rickettsiae*, and *Coxiella burnetii*, among others. A newly discovered *Ehrlichia* spp was reported to cause ehrlichiosis in Minnesota and Wisconsin; this *Ehrlichia* is closely related to *Ehrlichia muris* [262].

[m]Sensitivity of IFA antibody titers for tick-borne rickettsial diseases (RMSF, ehrlichiosis, and anaplasmosis) is dependent on the timing of specimen collection; the IFA is estimated to be 94%–100% sensitive after 14 days of onset of symptoms and sensitivity is increased if paired samples are tested.

[n]Treatment decisions for tick-borne rickettsial diseases for acutely ill patients should not be delayed while waiting for laboratory confirmation of a diagnosis. Fundamental understanding of signs, symptoms, and epidemiology of the disease is crucial in guiding requests for tests and interpretation of test results for ehrlichiosis, anaplasmosis, and RMSF. Misuse of specialized tests for patients with low probability of disease and in areas with a low prevalence of disease might result in confusion.

[o]Antibiotic therapy may diminish the development of convalescent antibodies in RMSF.

[p]Currently available serologic assays are designed specifically for *B. microti* and may not detect antibodies to other *Babesia* spp (eg, *B. duncani*, *B. divergens*).

[q]IgM antibodies develop 2 weeks after symptom onset.

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:

- Arthropod-borne diseases may be difficult to diagnose because signs and symptoms are generally nonspecific early in infection, including fever, chills, aches, pains, and rashes.
- Patient residence, travel history, recent exposure, and potential for tick bite are important.
- Serology remains the best tool for confirming the diagnosis of Lyme disease.
- NAATs are the preferred diagnostic modality for acute infection with *Anaplasma*, *B. miyamotoi*, *Babesia* spp, and *Ehrlichia* spp. *Babesia* may also be a microscopic diagnosis where available.
- Consultation with the microbiology laboratory is normally required to determine the specimens accepted, the available diagnostic assays, the location of the testing laboratory, and the turnaround time for results.

XIV. VIRAL SYNDROMES

This section will review commonly encountered viral infections in the United States, realizing that there are a myriad of viruses associated with human disease. Clinical microbiology laboratory tests that are commonly used to establish a diagnosis of viral infections are outlined below. Tests for HIV, EBV, CMV, VZV, HSV, human herpesvirus type 6 (HHV-6), enteroviruses, respiratory syncytial virus (RSV), influenza virus, adenovirus, lymphocytic choriomeningitis virus, BK virus, JC virus, hepatitis A to E viruses, parvovirus (erythrovirus) B19, measles, mumps, rubella, rabies virus, dengue virus, parechovirus, parainfluenza viruses, human metapneumovirus, West Nile virus (and other encephalitides), and Zika virus are specifically highlighted. Not all clinical microbiology laboratories provide the comprehensive services outlined in the tables below, especially in the case of serologic and molecular tests. When the recommended testing is not available in a local laboratory, it can often be referred to a reference or public health laboratory, although this approach may yield a delay in obtaining results.

Though an increasing number of molecular tests for infectious agents are gaining FDA clearance, many molecular assays for viral pathogens are LDTs, offered by CLIA-certified laboratories. Although LDTs require validation according to CLIA requirements prior to clinical use, performance may vary between laboratories. Throughout this section, the acronym NAAT generally refers to RT-PCR or real-time RT-PCR. Other specific techniques may be substituted with appropriate validation.

While molecular assays offer strong laboratory evidence for the presence of a viral agent, serologic tests may not be as conclusive. Notably, detection of IgM-class antibodies against a variety of viral agents may be associated with false-positive results [263]. Therefore, if the pretest probability of acute infection is low to moderate, it is good practice to measure IgG (or total IgG and IgM) antibodies at the time of presentation (acute phase) and 2–3 weeks later (convalescent phase) to assess for seroconversion, or if possible and depending on the assay format, to demonstrate a 4-fold or greater rise in antibody titers. False-positive results may also occur in assays measuring IgG-class antibodies, especially between closely related viruses (eg, flaviviruses). Additionally, maternal IgG antibodies readily cross the placenta and may confound laboratory results in neonates. Finally, the possibility of false-negative serologic results must also be recognized, particularly for patients who present soon after (~7 days) symptom onset or in patients who are significantly immunosuppressed. It is therefore important to carefully consider all patient factors, including age, co-circulating viruses, exposure and vaccination history, immunostatus, and timing of presentation when interpreting serologic results for diagnostic purposes.

Key points for the laboratory diagnosis of viral syndromes:

- Viral syndromes should be considered based on the patient's age, immune status, exposure and vaccination history, and many other variables.
- Viral yields are highest early in the infectious process.
- Samples should be obtained and tested for the most likely agents, with residual specimen being stored (preferably frozen) in the laboratory in case additional testing is necessary. Typically, it is not cost-effective to test initial samples broadly for numerous viruses.
- Sample collection and handling are essential components of obtaining a reliable viral test result; consult the microbiology laboratory to determine which specimens should be obtained and how to transport them to the laboratory.
- Many laboratories will not have broad virologic testing capabilities, requiring specimens to be referred externally and resulting in longer turnaround times for results.
- Antibody cross-reactivity among some closely related viral agents may result in nonspecific serologic results.

- Tests for immunity, previous viral infection (eg, for tissue donors), and new infections may have different assay formats, even when the same virus is being evaluated.

A. Human Immunodeficiency Virus

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 >10^5 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

Table 48. Laboratory Diagnosis of Human Immunodeficiency Virus Infection

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology (rapid, point-of-care tests)	HIV-1 and HIV-2 antibodies only HIV-1 and HIV-2 antigen and antibody combination	Oral fluid (saliva), Whole blood (finger stick, venipuncture)	Not applicable
Serology (laboratory-based tests)	HIV-1 and HIV-2 antibodies only HIV-1 and HIV-2 antigen and antibody combination HIV-1 and HIV-2 antibody differentiation	Plasma[a] Serum	EDTA or PPT, RT, ≤2 h Clot or SST, RT, ≤2 h
NAAT	HIV-1 DNA and RNA, qualitative HIV-2 DNA and RNA, qualitative HIV-1 RNA, quantitative (viral load) HIV-2 RNA, quantitative (viral load) HIV-1 genotypic or phenotypic drug resistance	Whole blood Plasma[b] Plasma	EDTA or citrate tube, RT, ≤2 h EDTA or PPT, RT, ≤2 h EDTA or PPT[a], RT, ≤2 h

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

[a]For viral load testing, blood collected in PPT should be processed within 6 hours of collection to separate plasma from cells prior to transport. Since polymerase chain reaction does not differentiate between such proviral DNA and cell-free viral RNA, leakage of proviral DNA from cells during storage in PPT may cause falsely elevated plasma HIV RNA level results.

[b]Nucleic acid amplification tests are commercially available to detect non-integrated HIV DNA present in cell-free plasma.

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

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 $\geq 0.5 \log_{10}$ 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

Table 49. Laboratory Diagnosis of Epstein-Barr Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology (include heterophile antibody test or Monospot)	Serum	Clot or SST, RT, ≤2 h
NAAT, qualitative	CSF	Sterile tube, RT, ≤2 h
NAAT, quantitative (viral load)	CSF	Sterile tube, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
	Whole blood, peripheral blood lymphocytes	EDTA or citrate tube, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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

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 RealTi*m*e 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

Table 50. Laboratory Diagnosis of Cytomegalovirus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Serum	Clot or SST, RT, ≤2 h
	CSF	Sterile tube, RT, <2 h
Antigenemia[a]	Whole blood	Heparin, EDTA, or citrate tube, RT, ≤2 h
Culture	Urine	Sterile container, RT, <2 h
NAAT, qualitative	Body fluids CSF Respiratory specimens Tissue Urine	Sterile container, RT, <2 h
NAAT, quantitative (viral load)	Plasma	EDTA or PPT, RT, ≤2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

[a]Direct counting of stained cells; method no longer considered optimal.

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

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

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

Table 51. Laboratory Diagnosis of Varicella Zoster Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
NAAT	Scraping of base of dermal lesion collected using swab	Viral transport medium[a], RT, <2 h
	CSF	Sterile tube, RT, <2 h
Serology[b]	Serum	Clot or SST, RT, ≤2 h
Direct fluorescent antibody test	Vesicle fluid on slide	Place in sterile container, RT, <2 h

Abbreviations: CSF, cerebrospinal fluid; NAAT, nucleic acid amplification test; RT, room temperature; SST, serum separator tube.

[a]M4 or M5 media acceptable; do not use calcium alginate-tipped swab; swab with wood shaft, or transport swab containing gel.

[b]Evaluation for anti–varicella zoster virus immunoglobulin M antibodies is not recommended as a means to establish recent or acute infection; NAAT or culture is preferred.

Table 52. Laboratory Diagnosis of Herpes Simplex Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
NAAT	Scraping of base of dermal or mucosal lesion collected using a swab	Place into viral transport medium[a], RT, <2 h
	CSF	Sterile tube, RT, <2 h
Serology[b]	Serum	Clot or SST, RT, ≤2 h
Direct fluorescent antibody test	Vesicle fluid on slide	Place in sterile container, RT, <2 h
Culture	Scraping of base of dermal or mucosal lesion collected using a swab	Place into viral transport medium[b], RT or on wet ice, <2 h

Abbreviations: CSF, cerebrospinal fluid; NAAT, nucleic acid amplification test; RT, room temperature; SST, serum separator tube.

[a]M4 or M5 media acceptable; do not use calcium alginate-tipped swab, wooden shaft swab, or transport swab containing gel.

[b]Evaluation for anti–herpes simplex virus immunoglobulin M antibodies is not recommended as a means to establish recent or acute infection; NAAT or culture is preferred.

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

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

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

Table 53. Laboratory Diagnosis of Human Herpesvirus Type 6 Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Serum	Clot or SST, RT, ≤2 h
NAAT	CSF	Sterile container, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
	Saliva	Sterile container, RT, <2 h
	Serum	SST, RT, ≤2 h
	Whole blood, PBMCs	EDTA or citrate tube, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PBMCs, peripheral blood mononuclear cells; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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

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

Similar to measles, mumps is considered eliminated in the United States, though travel-associated cases among unvaccinated

Table 54. Laboratory Diagnosis of Parvovirus (Erythrovirus) B19 Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Histopathology	Bone marrow	Sterile container, RT, <2 h Formalin container, RT, 2–24 h
Serology	Serum	Clot or SST, RT, ≤2 h
NAAT	Plasma	EDTA or PPT, RT, ≤2 h
	Serum	SST, RT, ≤2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Table 55. Laboratory Diagnosis of Measles (Rubeola) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	CSF	Sterile tube, RT, <2 h
	Serum	Clot or SST, RT, ≤2 h
Culture	CSF	Sterile tube, RT, <2 h
	Oropharyngeal or NP swab[a], nasal aspirate	Viral transport media, RT or on wet ice, <2 h
	Urine	Sterile container, RT, <2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h
NAAT	CSF	Sterile tube, RT, <2 h
	Oropharyngeal swab, oral fluid	Sterile container, RT, <2 h
	Urine	Sterile container, RT, <2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; NP, nasopharyngeal; RT, room temperature; SST, serum separator tube.
[a]Place the swab in viral transport medium, cell culture medium, or other sterile isotonic solution (eg, saline).

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

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

Table 56. Laboratory Diagnosis of Mumps Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	CSF	Sterile tube, RT, <2 h
	Serum	Clot or SST, RT, ≤2 h
Culture	CSF	Sterile tube, RT but best on wet ice, <2 h
	Oropharyngeal or NP swab[a]	Viral transport medium, RT, <2 h
	Parotid (Stensen) duct/buccal swab[b]	
	Urine[c]	Sterile container, RT but best on wet ice, <2 h
NAAT	CSF	Sterile tube, RT, <2 h
	Oropharyngeal or NP swab[a]	Viral transport medium, RT, <2 h
	Parotid (Stensen) duct/buccal swab[b]	
	Urine[c]	Sterile container, RT, <2 h

Abbreviations: CSF, cerebrospinal fluid; NAAT, nucleic acid amplification test; NP, nasopharyngeal; RT, room temperature; SST, serum separator tube.
[a]Place swab in viral transport medium, cell culture medium, or other sterile isotonic solution (eg, saline).
[b]Massage parotid gland for 30 seconds and then swab parotid (Stensen) duct using a viral culture transport swab.
[c]Specimen is associated with lower sensitivity for culture and NAAT.

Table 57. **Laboratory Diagnosis of Rubella**

Diagnostic Procedure	Optimal Specimen	Transport Issues and Optimal Transport Time
Serology	Serum	Clot or SST, RT, ≤2 h
NAAT	Oropharyngeal or nasopharyngeal swabs	Viral transport medium, RT, <2 h
	Urine	Sterile container, RT, <2 h

Abbreviations: NAAT, nucleic acid amplification test; RT, room temperature; SST, serum separator tube.

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

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

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).

Table 58. **Laboratory Diagnosis of BK Virus Infection**

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Cytology	Urine	100 mL urine in 250-mL clear plastic collection bottle containing 50 mL of 2% carbowax solution (Saccomanno fixative) or alternative fixative 50% ethyl alcohol in equal volume to urine, RT, <2 h
NAAT, quantitative (viral load)	Plasma	EDTA or PPT, RT, ≤2 h
	Serum	SST, RT, ≤2 h
	Urine	Sterile container, RT, <2 h

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Table 59. Laboratory Diagnosis of JC Virus Infection

Diagnostic Procedure	Optimal Specimen	Transport Issues and Optimal Transport Time
NAAT	CSF	Sterile tube, RT, <2 h

Abbreviations: CSF, cerebrospinal fluid; NAAT, nucleic acid amplification test; RT, room temperature.

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

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.

Table 60. Laboratory Diagnosis of Dengue Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Serum	Clot or SST, RT, <2 h
NS1 antigen	Serum	Clot or SST, RT, <2 h
NAAT	CSF	Sterile tube, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
	Serum	SST, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; NS1, nonstructural protein 1; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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

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

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

Table 61. Laboratory Diagnosis of Hepatitis A and E

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Hepatitis A virus IgM antibody	Plasma Serum	EDTA or PPT, RT, <2 h Clot or SST, RT, <2 h
	Hepatitis A virus IgG antibody		
	Hepatitis A virus total antibodies		
	Hepatitis E virus IgM antibody		
	Hepatitis E virus IgG antibody		
NAAT	Hepatitis A virus RNA, quantitative	Plasma Serum	EDTA or PPT, RT, ≤2 h SST, RT, ≤2 h
	Hepatitis E virus RNA, quantitative (viral load)		

Abbreviations: EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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

Table 62. Laboratory Diagnosis of Hepatitis B and D Viruses

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Hepatitis B surface antigen	Plasma Serum	EDTA or PPT, RT, <2 h Clot or SST, RT, <2 h
	Hepatitis B surface antibody		
	Hepatitis B core total antibodies		
	Hepatitis B core IgM antibody		
	Hepatitis B e antigen		
	Hepatitis B e antibody		
	Hepatitis D total antibodies		
	Hepatitis D IgM antibody		
	Hepatitis D IgG antibody		
	Hepatitis D antigen		
NAAT	Hepatitis B virus DNA, quantitative (viral load)	Plasma Serum	EDTA or PPT, RT, ≤2 h SST, RT, ≤2 h
	Hepatitis D virus RNA, quantitative (viral load)		

Abbreviations: EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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.

Table 63. Laboratory Diagnosis of Hepatitis C Virus

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	HCV IgG antibody screen	Plasma Serum	EDTA or PPT, RT, <2 h Clot or SST, RT, <2 h
	HCV IgG antibody confirmation		
NAAT	HCV RNA, qualitative	Plasma Serum	EDTA or PPT, RT, ≤2 h SST, RT, ≤2 h
	HCV RNA, quantification (viral load)		
	HCV genotyping		
	HCV NS3 resistance-associated substitutions (genotypes 1 and 3 only)		
	HCV NS5a resistance-associated substitutions (genotypes 1 and 3 only)		
	HCV NS5b resistance-associated substitution (genotypes 1 and 3 only)		

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HCV, hepatitis C virus; IgG, immunoglobulin G; NAAT, nucleic acid amplification test; NS, nonstructural protein; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

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

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

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

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

West Nile virus (WNV), alongside other endemic arboviruses including St Louis encephalitis, Lacrosse encephalitis, and California encephalitis viruses, can cause CNS infections.

Table 64. Laboratory Diagnosis of Enteroviruses and Parechoviruses Infection

Diagnostic Procedures	Optimal Specimen	Transport Issues and Optimal Transport Time
NAAT	CSF[a]	Sterile tube, RT, <2 h
	Plasma[b]	EDTA or PPT, ≤2 h
	Serum[b]	SST, RT ≤2 h
	Urine	Sterile container, RT, <2 h
Culture	Plasma[b]	EDTA or PPT, RT, <2 h
	Stool	Sterile container, RT, <2 h
	Throat swab	Sterile container or viral transport medium, RT, <2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

[a]A commercial US Food and Drug Administration–cleared product is available for rapid polymerase chain reaction testing for enteroviruses in CSF.

[b]Whole blood is a less reliable source for detection by culture or NAAT methods.

Table 65. Laboratory Diagnosis of Respiratory Syncytial Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
NAAT[a]	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT,<2 h
Antigen detection (direct fluorescent antibody stain or rapid immunoassay antigen detection method)	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Culture	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT or ideally on wet ice, <2 h

Abbreviations: NAAT, nucleic acid amplification test; NP, nasopharyngeal; RT, room temperature.

[a]Commercial products are available for rapid polymerase chain reaction testing for respiratory viruses.

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

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

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

Table 66. Laboratory Diagnosis of Influenza A and B Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
NAAT[a]	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Antigen detection (rapid)	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Culture	NP aspirate/washing, throat or NP swab, lower respiratory specimen	Sterile container or viral transport medium, RT or ideally on wet ice, <2 h

Abbreviations: NAAT, nucleic acid amplification test; NP, nasopharyngeal; RT, room temperature.

[a]US Food and Drug Administration–cleared commercial products are available for rapid NAAT testing for respiratory viruses.

Table 67. Laboratory Diagnosis of Infection With West Nile Virus and Other Endemic Arboviruses

Diagnostic Procedures	Optimal Specimens	Transport Issues and Optimal Transport Time
Serology	Serum	Clot or SST, RT, <2 h
NAAT[a]	CSF	Sterile tube, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
	Serum	SST, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

[a]NAATs for uncommon arboviruses (eg, California encephalitis viruses, LaCrosse encephalitis, St Louis encephalitis virus, Eastern equine encephalitis virus) are available through the Centers for Disease Control and Prevention or select public health laboratories.

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

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

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.

X. Parainfluenza

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

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

Table 68. Laboratory Diagnosis of Adenovirus Infection

Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
NAAT	NP aspirate/washing, throat or NP swab, lower respiratory specimen, stool, conjunctiva swab	Sterile container or viral transport medium, RT, <2 h
	CSF	Sterile tube, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
Antigen detection, rapid	NP swab, respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Antigen detection (adenovirus types 40 and 41)	Stool	Sterile container, RT, <2 h
Culture	NP aspirate/washing, throat or NP swab, lower respiratory specimen, stool, CSF	Sterile container or viral transport medium, RT, <2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; NP, nasopharyngeal; PPT, plasma preparation tube; RT, room temperature.

Table 69. Laboratory Diagnosis of Rabies Virus Infection

Diagnostic Procedure	Optimum Specimen	Transport Issues and Optimal Transport Time
Direct fluorescent antibody, histopathology	Nuchal skin biopsy, brain biopsy	Sterile container, RT, <2 h
Serology	CSF	Sterile tube, RT, <2 h
	Serum	Clot or SST, RT, ≤2 h
NAAT	Saliva	Sterile tube, RT, <2 h

See also Table 43.

Abbreviations: CSF, cerebrospinal fluid; NAAT, nucleic acid amplification test; RT, room temperature; SST, serum separator tube.

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

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.

Table 70. Laboratory Diagnosis of Lymphocytic Choriomeningitis Virus Infection

Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Serology	CSF	Sterile tube, RT, <2 h
	Serum	Clot or SST, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; RT, room temperature; SST, serum separator tube.

XV. BLOOD AND TISSUE PARASITE INFECTIONS

Blood and tissue parasites comprise a large number of protozoa and helminths found in both tropical and temperate climates worldwide [288–290]. Some parasitic infections are associated with high morbidity and mortality (eg, malaria, amebic encephalitis), whereas others cause only mild or asymptomatic disease (eg, filariasis due to *Mansonella* spp, toxoplasmosis in immunocompetent adults). As expected, the most commonly submitted specimens for laboratory identification of these parasites are whole blood, tissue aspirates/biopsies, and serum for serologic studies.

Microscopy remains the cornerstone of laboratory testing for the identification of most blood parasites and many tissue parasites [289–291]. Expert microscopic examination of Giemsa-stained thick and thin peripheral blood films is used for detection and identification of the protozoan blood parasites *Plasmodium*, *Babesia*, and *Trypanosoma*, and the filarial nematodes *Brugia*,

Table 71. Laboratory Diagnosis of Zika Virus Infection[a]

Diagnostic Procedures	Optimum Specimens	Transport Issues and Optimal Transport Time
Serology	CSF	Sterile tube, RT, <2 h
	Serum	Clot or SST, RT, ≤2 h
NAAT	CSF	Sterile container, RT, <2 h
	Plasma	EDTA or PPT, RT, ≤2 h
	Serum	SST, RT, ≤2 h
	Urine	Sterile tube, RT, <2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

Abbreviations: CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

[a]Additional specimens (eg, products of conception, tissue) may be validated for testing at select public health laboratories or the Centers for Disease Control and Prevention.

Wuchereria, *Loa loa*, and *Mansonella*, whereas microscopic examination, culture and/or nucleic acid amplification of ulcer samples, bone marrow, tissue aspirates, and biopsies are useful in the diagnosis of African trypanosomiasis, onchocerciasis, trichinosis, toxoplasmosis, and leishmaniasis. Although requiring a minimal amount of reagents and equipment, the accuracy of microscopic methods requires well-trained and experienced technologists. Even in the best hands, diagnosis may be hampered by sparseness of organisms on the slide and the subjective nature of differentiating similar-appearing organisms (*Plasmodium* vs *Babesia*; various microfilariae) or in identifying the species of *Plasmodium* present. The laboratory can enhance the sensitivity of these methods by employing a number of concentration procedures such as buffy coat examination, centrifugation, and filtration. In all of these procedures, samples must be properly obtained, transported to the laboratory as quickly as possible, and processed in a timely fashion to preserve organism viability and/or morphology. Organism viability and morphology may be adversely affected by a number of different factors including temperature, humidity, and exposure to fixatives or anticoagulants. Transportation requirements are described for each organism in the corresponding sections below.

Serologic assays for detection of antibodies are available as adjunctive methods for the diagnosis of a number of blood and tissue parasite infections. Unfortunately, none are sensitive or specific enough to be used to establish the diagnosis on their own. In particular, assays for infection with one helminth will often cross-react with antibodies to a different helminth [290]. When available, antibody titers may be used to determine the strength of the immune response or detect a trend in antibody levels over time. IFA can provide quantitative titer results but reading the slides is subjective and inherently prone to varying results. In contrast, EIAs typically provide only qualitative positive or negative results determined by an arbitrarily set breakpoint. Thus, clinicians will not be able to determine if a positive result was a very strong positive or a very weak one without calling the laboratory for more information. This can have important implications for interpretation of results that are not entirely consistent with the clinical picture. In some cases, it is desirable to confirm the result of an EIA by using a more specific immunoblot assay.

Laboratory methods that detect parasite antigens and/or nucleic acid provide an attractive alternative to traditional morphologic and serologic techniques. For example, a simple rapid immunochromatographic card assay for the detection of *Plasmodium* (BinaxNOW Malaria, Alere, Waltham, Massachusetts) has been cleared by the FDA for in vitro diagnostic use, and many more assays are commercially available for this purpose outside of the United States [292]. Rapid detection tests (RDTs) are particularly useful in acute care settings such as emergency departments or outpatient clinics to establish a diagnosis of malaria quickly while awaiting results of confirmatory blood films. These methods are also commonly used in smaller laboratories or during times (eg, night shift) when personnel with sufficient expertise to screen and interpret blood films for parasites. In general, most malaria RDTs, including the BinaxNOW Malaria, are adequately sensitive in typical patients with symptomatic malaria ("fever and chills") but lose sensitivity when the parasitemia is very low or infection is due to non-falciparum species [292]. Finally, the CDC [289] and a number of reference laboratories in the United States, Canada, and Europe perform extremely sensitive NAATs such as real-time PCR assays for certain blood and tissue parasites, including *Plasmodium*, *Babesia*, *Toxoplasma*, and the agents of amebic encephalitis. Clinicians should consult their microbiology laboratory to determine if their reference laboratory or other entity offers the desired testing. Molecular assays may be of particular use in patients with very low parasitemias or in specifically identifying organisms that cannot be differentiated microscopically. However, DNA may persist for days or weeks after successful treatment and detection does not necessarily correlate with the presence of viable organisms. In addition, the current restriction to the reference laboratory setting means that the time from specimen collection to receipt of result may be longer than desired for optimal patient care. In situations where infection is potentially life-threatening, initial testing should be performed locally and empiric treatment should be considered while awaiting results from the outside laboratory.

Key points for the laboratory diagnosis of blood and tissue parasites:

- Microscopy is the cornerstone of laboratory identification but is highly subjective and dependent on technologist experience and training.
- Proper specimen collection and transport are essential components of morphology and culture-based techniques.
- Serology shows significant cross-reactivity among helminths, including filaria.
- There are a limited number of antigen detection methods available for blood and tissue parasites in the United States.
- Automated hematology analyzers may fail to detect malaria or babesiosis parasites; request manual stain and evaluation if either agent is suspected.
- NAATs are useful for detection of low parasitemia or in specifically identifying organisms that cannot be differentiated microscopically.
- Antigen and nucleic acid detection methods should not be used to monitor response to therapy, since antigen or DNA may be detectable for days to weeks after successful treatment.
- NAATs for detecting blood and tissue parasites are currently available only from specialized laboratories and turnaround time may be prolonged.

Table 72 presents an inclusive overview of the approach to the diagnosis of blood and tissue parasitic infections based on published recommendations [288–290]. Important points are

Table 72. Approach to Diagnosis of Blood and Tissue Parasitic Infections

Disease/Organism	Main Diagnostic Test(s)	Remarks
Amebic meningitis/encephalitis due primarily to *Naegleria fowleri*, *Acanthamoeba* spp, and *Balamuthia mandrillaris* (free-living amebae)	Microscopy and culture of CSF or brain tissue	**Specimens for culture should not be refrigerated.** *Balamuthia mandrillaris* does not grow on standard agar (requires specialized cell culture). PCR from tissue or CSF is available from the CDC and some reference labs. Stained and unstained tissue slides may also be sent for identification of amebic trophozoites and/or cysts.
Angiostrongyliasis and Gnathostomiasis	Serology available at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (http://www.tm.mahidol.ac.th/en/tmhm/index-m.htm)	In eosinophilic meningitis, larvae may be rarely seen in CSF. Larvae may also be seen in tissue sections with associated eosinophils and/or necrosis.
Babesiosis due to *Babesia microti*, *Babesia divergens*, *Babesia duncani*, and *Babesia* sp MO-1 strain	Microscopy of Giemsa-stained thick and thin blood films	Real time PCR available from CDC and reference labs. **Most PCR assays detect *B. microti* only.** Serology does not distinguish between acute and past infection.
Baylisascaris encephalitis	Serology available from the CDC	Larvae may be seen on histopathologic sections of brain tissue.
Cysticercosis and echinococcosis	Serology from the CDC or referral laboratories. **Cross-reactivity may be observed between tests for either organism.**	Encysted larvae and/or hooklets can be seen in tissue biopsies or aspirates of cysts (echinococcosis).
Filariasis due to species of *Wuchereria*, *Brugia*, and *Mansonella*	Microscopy of Giemsa-stained thick and thin blood films. Examination of concentrated blood specimens (Knott, Nuclepore filtered blood, or buffy coat) increases sensitivity. Antibody and/or antigen detection EIA (*Wuchereria bancrofti* and *Brugia malayi*) in blood by the CDC or reference lab	**Blood films for *W. bancrofti* and *B. malayi* should be collected between 10 am and 2 pm when microfilariae are circulating. Repeat exams may be necessary due to low parasitemia.** Serology does not differentiate between these filariae.
Filariasis, onchocerciasis due to *Onchocerca volvulus*	Microscopy of "skin snip" after incubation in saline at 37°C.	**"Skin snips" should be from areas nearby nodules and should be "razor thin" with no visible blood.** Histopathologic examination of skin biopsy or resected nodule (onchocercoma) can identify microfilariae and/or adults. Serology from reference lab; does not differentiate between filaria.
Leishmaniasis, cutaneous due to various *Leishmania* spp	Microscopic exam of Giemsa-stained smears of biopsy touch impressions or aspirate from leading edge of ulcer; culture and PCR are available through the CDC (**contact CDC for collection kit prior to collecting biopsy**)	**Treatment is dependent on species identification (using culture or PCR) for disease acquired in South or Central America.** Histopathology is less sensitive than impression smears, culture, and PCR. **Serology is not useful for cutaneous disease.**
Leishmaniasis, visceral due to various *Leishmania* spp	Microscopic exam of Giemsa-stained bone marrow aspirate/ biopsy, splenic aspirate; culture, PCR and serology is available from the CDC (**contact CDC for collection kit prior to collecting biopsy**)	Positive rK39 serology is reported to be both sensitive and specific for the diagnosis of visceral leishmaniasis in various endemic areas of the world.
Malaria due to *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium knowlesi*	Stat microscopic examination of Giemsa-stained thick and thin blood films (**repeat testing every 12–24 h for a total of 3 exams before ruling out malaria**); rapid antigen detection tests followed by confirmatory blood films within 12–24 h	**Antigen tests lack sensitivity with low parasitemia and non-falciparum malaria and do not differentiate all species.** PCR from some reference laboratories will detect and differentiate all species. **Calculation of percentage parasitemia (using thick or thin blood films) is required for determining patient management and following response to therapy.**
Toxocariasis (visceral larva migrans)	Serology from CDC or referral laboratories	Larvae are only rarely seen in histopathologic sections of biopsies of liver or other infected tissues.
Toxoplasmosis due to *Toxoplasma gondii*	Serology (IFA, EIA, ELFA) from CDC or reference lab for detection of IgM and IgG. **Positive IgG seen in up to 15%–40% of US population due to previous exposure.** IgG avidity test and serial titers may distinguish between recent and past infection.	Cysts and tachyzoites can be seen in specimens from immunocompromised patients (eg, bronchoalveolar lavage, brain biopsy); PCR is available from some reference labs.
Trichinosis due to *Trichinella spiralis* and other species	Serology (EIA) from the CDC or reference lab	Encysted larvae can be seen in histopathologic sections of muscle biopsies.
Trypanosomiasis, African (African sleeping sickness) due to *Trypanosoma brucei gambiense* (West African) or *T. b. rhodesiense* (East African)	Microscopy of Giemsa-stained thick and thin blood films or buffy coat preps. **Parasitemia is often low, requiring repeated exams.** Aspirates of chancres and lymph nodes may also be examined. **Centrifuged CSF should be examined to evaluate for late stage disease. There is an infection hazard from live organisms in blood specimens.**	Plasma cells with large eosinophilic antibody globules may be seen in CSF and brain biopsy. Card agglutination test for trypanosomiasis is available in endemic settings for detection of *T. b. gambiense* infection. Contact the CDC or Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium (http://www.itg.be/E/card-agglutination-test-for-human-trypanosomiasis).
American trypanosomiasis (Chagas disease) due to *Trypanosoma cruzi*	Microscopy of Giemsa-stained thick and thin blood films or buffy coat preps. Serology available through the CDC. **There is an infection hazard from live organism in blood specimens.**	**Parasitemia is very low in chronic infection.** IgG antibody may persist for decades and its presence is considered evidence of chronic infection. An FDA-approved test is available for screening blood donors but is not available for diagnostic purposes.

Sources: [288, 290–292, 295].

"CDC" refers to the Division of Parasitic Diseases at the CDC (https://www.cdc.gov/dpdx/). "Reference Labs" refers to any laboratory that performs esoteric testing not usually done in routine hospital laboratories.

Abbreviations: CDC, Centers for Disease Control and Prevention; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; ELFA, enzyme-linked fluorescence antibody; FDA, US Food and Drug Administration; HRP2, histidine-rich protein 2; IFA, immunofluorescence assay; IgG, immunoglobulin G; IgM, immunoglobulin M; PCR, polymerase chain reaction.

bolded. Subsequent sections A and B provide more detailed information on the diagnosis of parasitic infections that are of particular concern to practitioners in North America (babesiosis and American trypanosomiasis) or in which rapid and accurate diagnosis is crucial because of the life-threatening nature of the infection (malaria and babesiosis). With all testing, it is important to note that results are only as reliable as the experience, resources, and expertise of the laboratory performing the tests. In general, large public health and reference laboratories are more likely than community laboratories to have the experience and volume of specimens to properly validate the more esoteric tests. Direct communication by phone or email will sometimes hasten specimen processing and result reporting from public health laboratories, especially when there is an urgent clinical situation. The DPDx website at the CDC (https://www.cdc.gov/dpdx/diagnosticprocedures/index.html) provides a list of currently available diagnostic tests for parasitic infections available from the CDC. The CDC also provides a valuable telediagnostic consultation service that can be accessed through the DPDx website for both the laboratorian and clinician. The availability of rapid shipping methods (FedEx, United Parcel Service, United States Postal Service, etc) and email or other electronic communication allow reporting of results from specialty laboratories, including those in Europe and Asia, in surprisingly short periods of time. It is useful to obtain shipping information from such laboratories to avoid unnecessary delays because of customs or airline regulations or other delivery problems.

A. Babesia and Malaria

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

Table 73. Summary of Laboratory Detection Methods for Babesiosis and Malaria Infection

Diagnostic Procedure	Optimum Specimen	Transport Considerations	Estimated TAT[a]
Microscopy of Giemsa stained thick and thin blood films with determination of percentage parasitemia	Drop of blood from finger stick or venipuncture needle placed directly on glass slides and blood films made immediately	Slides should be made from blood within 1 h. Prolonged exposure to EDTA can alter parasite morphology. Thick blood films dry slowly and should be protected from inadvertent smearing or spillage and dust. Use of the "scratch" method will improve adherence and allow for examination as soon as the blood is visibly dry.	2-4 h
QBC centrifugal system	Buffy coat concentrate of RBCs from venous blood in acridine orange containing capillary tubes	QBC concentrates and slides should be made from blood within 1 h for optimal preservation of parasite morphology	2-4 h
Antigen detection immunochromatographic assay (generally termed rapid diagnostic test)	Drop of blood from finger stick or venipuncture needle placed directly on rapid diagnostic test pad	Test should be performed as soon as possible but blood may be stored at 2°C–30°C for up to 3 d for some commercial assays.	15–30 min
Serologic detection of antibody to *Babesia microti* and *Plasmodium* spp	1.0 mL of serum from clotted blood tube	Serum should be separated from blood within several hours. Store serum refrigerated or frozen if not tested within 4–6 h to preserve antibody and prevent bacterial growth. Avoid use of hyperlipemic or hemolyzed blood.	4–6 h
NAAT	Typically 1.0 mL venipuncture blood in EDTA tube	Test should be performed as soon as possible, but blood may be transported refrigerated over 48 hours	1–2 h

Sources: [6, 7].

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NAAT, nucleic acid amplification test; QBC, quantitative buffy coat; RBC, red blood cell; TAT, turnaround time.

[a]Transportation time and nucleic acid extraction time are not included in this estimate.

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*

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].

Table 74. Summary of Tests for Trypanosomes

Diagnostic Procedures	Optimum Specimen	Transport Considerations	Estimated TAT[a]
Microscopy of Giemsa-stained thick and thin peripheral blood films in fresh and stained preparations.	Drop of blood from finger stick or venipuncture needle placed directly on glass slides and blood films made immediately OR Buffy coat concentrate from anticoagulated venous blood (thin smear or fresh wet prep for motile organisms)	Slides and wet preps should be made from blood within 1 h. Thick blood films dry slowly and should be protected from inadvertent smearing or spillage and dust. Use of the "scratch" method will improve adherence and allow for examination as soon as the blood is visibly dry.	2–4 h
Microscopic examination of tissue aspirates/biopsies by Giemsa/H&E stains	Fluid from needle aspirate of enlarged lymph nodes or tissue biopsies from lymph nodes, skin lesions, heart, GI tract, or other organ	Fresh aspirated fluid should be stained and examined as soon as possible, preferably within 1 h of sampling.	2 h–3 d
Culture in NNN or other suitable media with subsequent microscopic examination for motile trypanosomes	Anticoagulated blood or buffy coat, tissue aspirates, and tissue biopsies	Fresh specimens should be inoculated into culture medium as soon as possible, preferably within 1 h of collection for preservation of organism viability.	2–6 d
Serology	1.0 mL of serum from clotted blood. Plasma is also acceptable for the Ortho donor test.	Serum or plasma should be separated from blood within several hours. Store serum refrigerated or frozen if not tested within 4–6 h to preserve antibody and prevent bacterial growth. Avoid use of hyperlipemic or hemolyzed blood.	1 d
NAAT	Typically 1.0 mL venipuncture blood in EDTA tube	Test should be performed as soon as possible, but blood may be transported refrigerated over 48 hours.	1–2 h

Abbreviations: EDTA, ethylenediaminetetraacetic acid; GI, gastrointestinal; H&E, hematoxylin & eosin; NAAT, nucleic acid amplification test; NNN, Novy-MacNeal-Nicolle; TAT, turnaround time.
[a]Transportation time and nucleic acid extraction time are not included in this estimate.

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.

Notes

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