

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)^a

Ellen Jo Baron,^{1,2} J. Michael Miller,³ Melvin P. Weinstein,⁴ Sandra S. Richter,⁵ Peter H. Gilligan,⁶ Richard B. Thomson Jr.,⁷ Paul Bourbeau,⁸ Karen C. Carroll,⁹ Sue C. Kehl,¹⁰ W. Michael Dunne,^{11,12} Barbara Robinson-Dunn,¹³ Joseph D. Schwartzman,¹⁴ Kimberle C. Chapin,¹⁵ James W. Snyder,¹⁶ Betty A. Forbes,¹⁷ Robin Patel,¹⁸ Jon E. Rosenblatt,¹⁸ and Bobbi S. Pritt¹⁸

¹Department of Pathology, Stanford University School of Medicine, Stanford, California; ²Cepheid, R&D, Sunnyvale, California; ³Microbiology Technical Services, LLC, Dunwoody, Georgia; ⁴Department of Medicine and Pathology, Robert Wood Johnson Medical School, New Brunswick, New Jersey; ⁵Department of Clinical Pathology, Cleveland Clinic, Cleveland, Ohio; ⁶Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina; ⁷Department of Pathology, NorthShore University HealthSystem, Evanston, Illinois; ⁸Scientific Affairs, BD Diagnostics, Sparks, Maryland; ⁹Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland; ¹⁰Department of Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin; ¹¹bioMérieux, Inc, Durham, North Carolina, and ¹²Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri; ¹³Department of Pathology, William Beaumont Hospital to Beaumont Health System, Royal Oak, Michigan; ¹⁴Department of Pathology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire; ¹⁵Department of Pathology, Brown Alpert Medical School, Providence, Rhode Island; ¹⁶Department of Laboratory Medicine, University of Louisville, Kentucky; ¹⁷Department of Pathology, Virginia Commonwealth University Medical Center, Richmond, Virginia; and ¹⁸Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

The critical role of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician and the microbiologists who provide enormous value to the health care team. This document, developed by both laboratory and clinical experts, provides information on which tests are valuable and in which contexts, and on tests that add little or no value for diagnostic decisions. Sections are divided into anatomic systems, including Bloodstream Infections and Infections of the Cardiovascular System, Central Nervous System Infections, Ocular Infections, Soft Tissue Infections of the Head and Neck, Upper Respiratory Infections, Lower Respiratory Tract infections, Infections of the Gastrointestinal Tract, Intraabdominal Infections, Bone and Joint Infections, Urinary Tract Infections, Genital Infections, and Skin and Soft Tissue Infections; or into etiologic agent groups, including Tickborne 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. There is redundancy among the tables and sections, as many agents and assay choices overlap. The document is intended to serve as a reference to guide physicians in choosing tests that will aid them to diagnose infectious diseases in their patients.

Keywords. laboratory diagnosis; microbiology testing; specimen processing; physician-laboratory communication; medical laboratories.

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^aAlthough accurate and authoritative, IDSA considers adherence to the recommendations in this guide to be voluntary, with the ultimate determination regarding their application to be made by the physician in the light of each patient's individual circumstances.

Correspondence: Ellen Jo Baron, PhD, Cepheid, R&D, 1315 Chesapeake Terrace, Sunnyvale, CA 94089, USA (ejbaron@stanford.edu).

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Common abbreviations used throughout the text:

CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; GI, gastrointestinal; IFA, indirect fluorescent antibody; IIF, indirect immunofluorescence; MRSA, methicillin-resistant *Staphylococcus aureus*; NAAT, nucleic acid amplification test; PMN, polymorphonuclear neutrophil; RPR, rapid plasma reagin (test for syphilis); RT, room temperature; VRE, vancomycin-resistant enterococcus; WBC, white blood cell

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. Prokaryotic microorganisms, while genetically less complex than multicellular eukaryotes, are 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, so a specialist in microbiology is needed to facilitate microbiology laboratory result interpretation. Clearly, all microbes grow, multiply, and die very quickly. If any of those events occur during specimen collection, transport, or storage, the results of analysis will be compromised and interpretation could be misleading. Therefore, attention to preanalytical specimen management in microbiology is critical to accuracy.

Physicians 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. In order 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 as well as what the laboratory needs, including ensuring that specimens arrive at the laboratory for analysis as quickly as possible after collection (Introduction-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 very different information. The microbiology laboratory needs a specimen that has been appropriately selected, collected, and transported to the

Table Introduction-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 (2nd choice) – flocced 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 (2nd choice) – flocced 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 (2nd 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 – flocced swabs are recommended	Virus swab transport device, RT, 2 h
Suspected agent of bioterrorism	Refer to Centers for Disease Control and Prevention website: http://emergency.cdc.gov/documents/PPTResponse/table2specimensselection.pdf	
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	Closed container, RT, 2 h

Abbreviations: AFB, acid-fast bacillus; NAAT, nucleic acid amplification test; RT, Room Temperature.

^a Contact the microbiology laboratory regarding appropriate collection and transport devices and procedures since transport media such as Cary-Blair or parasite preservative transport for stool specimens, boric acid for urines, 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.

laboratory for analysis. Caught in the middle, between the physician and laboratory, are those who 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 laboratorian specialist. This document illustrates 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 (ABMM), the American Board of Pathology (ABP), or the American Board of Medical Laboratory Immunology (ABMLI) 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 staff, 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, it impacts patient length of stay, hospital costs, and laboratory costs, and influences laboratory efficiency. Clinicians should consult the laboratory to ensure that selection, collection, transport, and storage of patient specimens are performed 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 are:

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,” thus providing irrelevant information that could result in inaccurate diagnosis and inappropriate therapy.
3. “Background noise” must be avoided where possible. Many body sites have normal microbiota that can easily contaminate the specimen. 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), 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 nasopharyngeal and 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. These manuals are usually supported by the literature.

6. A specimen should be collected prior to administration of antibiotics. Once antibiotics have been started, the flora changes, leading to potentially misleading culture results.

7. Susceptibility testing should be performed 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 be allowed to 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 staff 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 in order to establish or confirm a diagnosis.

Welcome and 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

The full text of this document, available online, is organized by body system, although many organisms are capable of causing disease in more than one 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. Another unique feature is that in most sections, there are targeted recommendations and precautions regarding selecting and collecting specimens for analysis for a disease process. Within each section, 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 (RT) 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 Update

The document has been endorsed by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Future modifications are to be expected, as diagnostic microbiology is a dynamic and rapidly changing discipline.

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 results within 48 hours; incubation for more than 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* 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, *Candida* spp tend to 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 I-1 below 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 whippelii*). 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 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 more than 2 bottles depending on the system. For children, an age- and weight-appropriate volume of blood should be cultured (see Table I-1a for recommended volumes). A second important determinant is the number of blood culture sets performed during a given septic episode. Generally, in adults with a suspicion of BSI, 2–4 blood culture sets should be obtained in the evaluation of each septic episode [5, 7].

The timing of blood culture orders should be dictated by patient acuity. In urgent situations, 2 or more blood culture sets can be obtained sequentially over a short time interval, after which empiric therapy can be initiated. In less urgent situations, obtaining blood culture sets may be spaced over several hours or more.

Contaminated 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 flora, meticulous care should be taken in skin preparation prior to venipuncture. 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 (data summarized in refs [1] and [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]. CHG is not recommended for use in infants less than 2 months of age.

Table I-1. Blood Culture Laboratory Diagnosis Organized by Etiologic Agent

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
<i>Staphylococcus</i> spp <i>Streptococcus</i> spp, <i>Enterococcus</i> spp <i>Listeria monocytogenes</i> <i>Enterobacteriaceae</i> <i>Pseudomonas</i> spp <i>Acinetobacter</i> spp HACEK ^c bacteria <i>Brucella</i> spp Anaerobic bacteria	Adults: 2–4 blood culture sets per septic episode Infants and children: 2 or more blood culture sets	20–30 mL of blood per culture set in adults injected into at least 2 blood culture bottles ^a Blood volume depends on the child's weight (see Table in footnote 3) ^b	Inoculated culture vials should be transported to the Laboratory as soon as possible (ASAP) at RT, organisms will usually survive in inoculated culture vials even if not incubated immediately
<i>Bartonella</i> spp	2 or more lysis- centrifugation (Isolator) blood culture vials ^d NAAT	10 mL of blood should be inoculated directly into each lysis- centrifugation culture vial 5 mL of plasma	Lysis-centrifugation culture vials should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation EDTA tube, RT, 2 h
<i>Legionella</i> spp	2 or more lysis- centrifugation (Isolator) blood culture vials ^e <i>Legionella</i> urine antigen test (for serotype 1)	10 mL of blood should be inoculated directly into each lysis- centrifugation culture vial 10 mL of mid-stream, clean-catch urine ^f	Lysis-centrifugation culture vials should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation Closed container, RT, 2 h
<i>Coxiella burnetii</i>	Coxiella IFA serology NAAT	5 mL of serum 5 mL of plasma	Clot tube, RT, 2 h EDTA tube, RT, 2 h
<i>Tropheryma whipplei</i>	NAAT	5 mL of plasma	EDTA tube, RT, 2 h
Yeast	Adults: 2–4 blood culture sets (see above) Infants and children: 2 or more blood culture sets (see above)	20–30 mL of blood per culture in adults injected into at least 2 blood culture bottles ^g As much blood as can be conveniently obtained from children; volume depends on weight of child (see following table) ^b	Inoculated culture vials should be transported ASAP at RT to the laboratory for early incubation Organisms will usually survive in inoculated culture vials even if not incubated immediately. <i>Malassezia</i> spp require lipid supplementation; lysis-centrifugation is recommended for their recovery.
Filamentous and dimorphic fungi ^h	2 or more 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 bacillus; IFA, indirect fluorescent antibody; NAAT, nucleic acid amplification test; RT, room temperature.

^a 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.

^b Recommended volumes of blood for culture in pediatric patients (Table I-1a) [1].

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

^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 Because 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, MD]) 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.

Table I-1a. 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.

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 work-up and reporting of common blood culture contaminants (eg, coagulase-negative staphylococci, viridans group streptococci, diphtheroids, *Bacillus* species 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 work-up and susceptibility results.

Physicians should expect to be called and notified by the laboratory every time a blood culture becomes positive because 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).
- 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 2–3 bottle blood culture set for adults, at least one aerobic and one anaerobic; use 1–2 aerobic bottles for children.
- *Streptococcus pneumoniae* and some other gram-positive organisms may grow best in the anaerobic bottle.

B. Infections Associated With Vascular Catheters

The diagnosis of catheter-associated BSIs often is 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 [8, 9]. 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.

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 (IV) catheter tips at the time of catheter removal has no clinical value and should not be done [10]. 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 blood culture system. If both BCs grow the same organism and the BC drawn from the device becomes positive more than 2 hours before the BC drawn by venipuncture, there is a high probability of catheter-associated BSI [11].
- 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 [12].

- Catheter tip or segment cultures: The semiquantitative method of Maki et al [10] is used most commonly; interpretation requires an accompanying peripheral blood culture. 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 I-2).

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 I-3 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 (CNS) INFECTIONS

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

Organisms usually enter the central nervous system 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 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.

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

Table I-2. Laboratory Methods for Diagnosis of Infected Aneurysms and Vascular Grafts

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

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

^a 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.

^b If aerobic bacteria are suspected. If anaerobes are suspected, then the culture should consist of an aerobic and anaerobic bacterial culture.

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

Table I-3. Laboratory Diagnosis of Pericarditis and Myocarditis

Etiologic Agents ^a	Diagnostic Procedures	Optimum Specimens	Transport Issues; 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 I-A above)	Pericardial fluid or pericardium biopsy	Sterile container, RT, 2 h
Mycobacteria	Acid fast smear AFB culture Blood cultures (see I-A above)	Pericardial fluid or pericardium biopsy ^c	Sterile container, RT, 2 h
Viruses			
Coxsackie B virus Coxsackie A virus	Virus-specific serology	Acute and convalescent sera	Clot tube, RT, 2 h
Echovirus Polio virus	Virus-specific NAAT (may be first choice if test is available)	Pericardial fluid or pericardium biopsy	Closed container, RT, 2 h
Adenovirus HIV	Virus culture (culture not productive for all virus types)	Pericardial fluid or pericardium biopsy	Virus transport device, on ice, immediately
Mumps virus Cytomegalovirus Other viruses	Histopathologic examination	Pericardial fluid or pericardium biopsy	Place in formalin and transport to histopathology laboratory for processing.
Parasites^d			
<i>Trypanosoma cruzi</i>	Parasite-specific serology	Acute and convalescent sera	Clot tube, RT, 2 h
<i>Trichinella spiralis</i>	Blood smears ^e	5 mL of peripheral blood	EDTA tube, RT
<i>Toxoplasma gondii</i>	Histopathologic examination <i>Toxoplasma</i> 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 bacillus; 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 (*R. rickettsii*, *C. burnetii*), chlamydiae, *B. burgdorferi*, *T. pallidum*, *Nocardia* spp, *T. whipplei*, *L. pneumophila*, *Actinomyces* spp, *E. histolytica*, *Ehrlichia* spp, *T. 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 CDC 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.

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. NAATs for most agents are not commercially available, so only laboratory-developed tests can be used, with variable sensitivities and specificities. 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 central nervous system infections:

- Whenever possible, collect specimens prior to initiating antimicrobial therapy.
- Two to four blood cultures should also be obtained if bacterial meningitis is suspected.
- Inform the Microbiology Laboratory if unusual organisms are possible (such as *Nocardia*, fungi, mycobacteria, etc.), for which special procedures are necessary.
- Do not refrigerate cerebrospinal fluid.
- 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 enteroviruses (primarily echoviruses and coxsackieviruses) and bacteria (*Streptococcus pneumoniae* and *Neisseria meningitidis*; Table II-1). Patient age and other factors (ie, immune status, post neurosurgery, trauma) are associated with specific bacterial pathogens.

Molecular testing has replaced viral culture for the diagnosis of enteroviral meningitis, but is not routinely available for the detection of bacteria in CSF. 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 [13]. Bacterial antigen testing on CSF is not recommended but may have some value in patients who received therapy prior to specimen collection with negative Gram stain and negative culture results [14]. 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 ≥ 4 weeks) include *Mycobacterium tuberculosis*, fungi, and spirochetes (Table II-1). Because the sensitivity of nucleic acid amplification tests (NAAT) for *M. tuberculosis* in nonrespiratory specimens may be poor, culture should also be requested [15]. The reported sensitivity of culture for diagnosing tuberculous meningitis is 25%–70% [16]. The highest yields for acid fast bacillus (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 *C. neoformans* or *C. 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 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 [17]. Immune status, travel, and other exposure history (insects, animals, water, sexual) should guide testing. IDSA practice guidelines provide a detailed listing of risk factors associated with specific etiologic agents [18].

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 $>95\%$; HSV is cultured from CSF in $<5\%$ of cases [19, 20]. 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) [19]. 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 II-2).

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, intraabdominal, esophageal, endocarditis). A brain abscess in an immunocompetent host is usually caused by bacteria (Table II-3). A wider array of organisms is encountered in immunocompromised individuals.

D. Central Nervous System Shunt Infections

Shunts are placed to divert cerebrospinal fluid 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. In total, 5%–15% of shunts become infected (Table II-4). 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.

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,

Table II-1. Laboratory Diagnosis of Meningitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacterial			
<i>Streptococcus pneumoniae</i>	Gram stain ^a	Cerebrospinal fluid, blood	Sterile container (CSF), aerobic blood culture bottle (blood), RT, immediately
<i>Neisseria meningitidis</i>	Aerobic bacterial culture		
<i>Listeria monocytogenes</i>			
<i>Streptococcus agalactiae</i>			
<i>Haemophilus influenzae</i>			
<i>Escherichia coli</i>			
Other <i>Enterobacteriaceae</i>			
<i>Elizabethkingia meningoseptica</i>			
<i>Mycobacterium tuberculosis</i>	AFB smear	Cerebrospinal fluid (≥5 mL)	Sterile container, RT; 2 h
	AFB culture		
	<i>M. tuberculosis</i> NAAT ^b	Cerebrospinal fluid	Closed container, RT, 2 h
Spirochetal			
<i>Treponema pallidum</i> (syphilis)	VDRL, FTA-ABS	Cerebrospinal fluid	Sterile container, RT, 2 h
	Traditional: RPR screening test with positive RPR confirmed by <i>T. pallidum</i> particle agglutination (TP-PA) test or other treponemal confirmatory test	1 mL serum	Clot tube, RT, 2 h
	Reverse sequence: EIA or chemiluminescent immunoassay treponemal screening test with positive confirmed by RPR (negative RPR reflexed to TP-PA)		
<i>Borrelia burgdorferi</i> (Lyme disease)	<i>B. burgdorferi</i> antibodies, IgM and IgG with Western blot assay confirmation (not validated for CSF)	1 mL serum	Clot tube, RT, 2 h
		1 mL CSF (include a CSF index: simultaneous CSF:serum ratio of <i>B. burgdorferi</i> antibodies with normalized protein amounts).	
	<i>B. burgdorferi</i> antibodies, IgM and IgG with Western blot assay confirmation (not validated for CSF)	Cerebrospinal fluid	Closed container, RT, 2 h
	<i>B. burgdorferi</i> NAAT (low sensitivity)	Cerebrospinal fluid	Closed container, RT, 2 h
<i>Leptospira</i> species	<i>Leptospira</i> culture (special media required; rarely available in routine laboratories)	1st week of illness: Cerebrospinal fluid, 10 mL blood	Sterile container, heparin or citrate tube (blood), RT, immediately
		After 1st week of illness: 10 mL urine (neutralized)	Sterile container, RT, immediately
	<i>Leptospira</i> antibody, microscopic agglutination test	1 mL serum	Clot tube, RT, 2 h
Fungal			
<i>Cryptococcus neoformans</i> , <i>Cryptococcus gattii</i>	<i>Cryptococcus</i> antigen test	Cerebrospinal fluid	Closed container, RT, 2 h
	Gram stain	Cerebrospinal fluid	Sterile container, RT, 2 h
	Aerobic bacterial culture (faster growth on blood agar medium)		
	Fungal culture		
<i>Coccidioides</i> species ^c	<i>Coccidioides</i> antibody, complement fixation and immunodiffusion ^c	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Calcofluor stain	Cerebrospinal fluid	Sterile container, RT, 2 h
	Fungal culture		

Table II-1 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Parasitic			
<i>Acanthamoeba</i> spp	See Table II-2 – Encephalitis		
<i>Naegleria fowleri</i>			
Viral			
Enteroviruses (nonpolio)	Enterovirus NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Parechoviruses	Parechovirus NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Herpes simplex virus (HSV)	HSV 1 and 2 NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Varicella zoster virus (VZV)	VZV NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Lymphocytic choriomeningitis virus (LCM)	LCM antibodies, IgM and IgG, IFA	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Mumps culture and Mumps NAAT	Cerebrospinal fluid, urine, buccal swab	Sterile container, on ice, immediately Viral transport device, on ice, immediately
Human immunodeficiency virus (HIV)	^d		

Abbreviations: AFB, acid-fast bacillus; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; FTA, fluorescent treponemal antibody; 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; TP-PA, *T. pallidum* particle agglutination; 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 *M. tuberculosis*.

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

^d 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 – VIRAL SYNDROMES; HIV diagnosis).

anaerobes, often polymicrobial; Table II-5). Mycobacteria and fungi are rare causes. Predisposing conditions include sinusitis, otitis media, mastoiditis, neurosurgery, head trauma, subdural hematoma, and meningitis (infants).

The pathogenesis of spinal epidural abscess includes hematogenous spread (skin, urinary tract, mouth, mastoid, lung infection), direct extension (vertebral osteomyelitis, discitis), trauma, or post-procedural 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 (MRI) 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 [26].

Table II-2. Laboratory Diagnosis of Encephalitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Viral			
Herpes simplex virus (HSV)	HSV 1 and 2 NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Enteroviruses (nonpolio)	Enterovirus NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Parechoviruses	Parechovirus NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
West Nile virus (WNV)	WNV IgM antibody ^a	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	WNV NAAT ^b	Cerebrospinal fluid and/or 1 mL serum	Closed container, RT, 2 h
Other arboviruses ^c	Virus specific antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
Varicella-zoster virus (VZV) ^d	VZV NAAT	Cerebrospinal fluid or 1 mL plasma	Closed container or EDTA tube (blood), RT, 2 h
	VZV antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
Epstein-Barr virus (EBV)	EBV NAAT ^e	Cerebrospinal fluid or 1 mL plasma	Closed container or EDTA tube (blood), RT, 2 h
	EBV antibodies, VCA IgG and IgM, EBNA	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
Cytomegalovirus (CMV) ^f	CMV NAAT ^g	Cerebrospinal fluid or 1 mL plasma	Closed container or EDTA tube (blood), RT, 2 h
	CMV antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
Human herpes virus 6 (HHV-6)	HHV-6 NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
JC virus	JC virus NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Mumps culture	Cerebrospinal fluid, urine	Sterile container, on ice, immediately
	Mumps NAAT		
	Mumps NAAT	Buccal swab	Viral transport device, on ice, immediately
Measles (Rubeola) virus	Measles antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Measles culture and Measles NAAT	Cerebrospinal fluid, 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	Cerebrospinal fluid or 1 mL plasma	Closed container or EDTA (blood), 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	Cerebrospinal fluid and 1 mL serum	Closed container, clot tube (blood), RT, 2 h
Lymphocytic choriomeningitis virus (LCM)	LCM antibodies, IgM and IgG, IFA	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube, RT (blood), 2 h
Bacterial			
<i>Mycobacterium tuberculosis</i>	See Table II-1 - Meningitis		
<i>Bartonella</i> spp	<i>Bartonella</i> spp NAAT	Cerebrospinal fluid or plasma	Closed container or EDTA (blood), RT, 2 h
	<i>Bartonella</i> spp antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h

Table II-2 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Mycoplasma pneumoniae</i>	<i>M. pneumoniae</i> NAAT	Cerebrospinal fluid or respiratory	Closed container, RT, 2 h
	<i>M. pneumoniae</i> antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
<i>Tropheryma whipplei</i> (Whipple's Disease)	<i>Tropheryma whipplei</i> NAAT	Cerebrospinal fluid	Closed container, RT, 2 h
<i>Listeria monocytogenes</i>	Gram stain	Cerebrospinal fluid, blood	Sterile container, aerobic blood culture bottle, RT, 2 h
	Aerobic bacterial culture		
	<i>Listeria</i> antibody, CF	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
<i>Coxiella burnetii</i> (Q fever)	<i>C. burnetii</i> antibodies, IgM and IgG	1 mL serum	Clot tube, RT, 2 h
	<i>C. burnetii</i> NAAT	Whole blood	EDTA tube, RT, 2 h
		Tissue	Closed container, RT, 2 h
<i>Rickettsia rickettsii</i> (Rocky Mountain spotted fever, RMSF), <i>R. typhi</i>	<i>Rickettsia</i> spp antibodies, IgG and IgM, IFA	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	<i>R. rickettsii</i> DFA or IHC and NAAT	Skin biopsy from rash	Closed container, RT, 2 h
	<i>R. rickettsii</i> NAAT	Whole blood	EDTA tube, RT, 2 h
<i>Ehrlichia chaffeensis</i> , <i>Anaplasma phagocytophilum</i>	<i>E. chaffeensis</i> and <i>A. phagocytophilum</i> antibodies, IgM and IgG	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	<i>E. chaffeensis</i> and <i>A. phagocytophilum</i> NAAT	Whole blood	EDTA tube, RT, 2 h
Other: <i>B. burgdorferi</i> , <i>T. pallidum</i> , <i>Leptospira</i> spp	See Table II-1 - Meningitis		
Fungal			
<i>Cryptococcus neoformans</i> , <i>Cryptococcus gattii</i>	Cryptococcus antigen test	Cerebrospinal fluid, 1 mL serum	Closed container, clot tube (blood), RT, 2 h
	Gram stain	Cerebrospinal fluid	Sterile container, RT, 2 h
	Aerobic bacterial culture		
	Fungal culture		
<i>Coccidioides</i> species	<i>Coccidioides</i> antibody, immunodiffusion and complement fixation	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Calcofluor stain	Cerebrospinal fluid, other sites	Sterile container, RT, 2 h
	Fungal culture		
	Histologic examination	Tissue or formalin-fixed tissue	Sterile container, RT, 2 h or formalin, indefinite
Parasitic			
<i>Acanthamoeba</i> spp	Microscopic wet mount	Cerebrospinal fluid	Closed container, RT, 2 h
<i>Naegleria fowleri</i>	Giemsa stain		
	Histology (trichrome stain)	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h
	Culture	Cerebrospinal fluid, brain tissue	Sterile container, RT, 2 h
	<i>Acanthamoeba</i> antibody IFA ⁱ	1 mL serum	Clot tube, RT, 2 h
	<i>Acanthamoeba</i> IIF staining ⁱ	Brain tissue	Closed container, RT, 2 h
<i>Balamuthia mandrillaris</i>	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h
	<i>Balamuthia</i> antibody, IFA ⁱ	1 mL serum	Clot tube, RT, 2 h
	<i>Balamuthia</i> IIF staining ⁱ	Brain tissue	Closed container, RT, 2 h
<i>Baylisascaris procyonis</i> ^j	<i>B. procyonis</i> antibodies	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
<i>Trypanosoma brucei</i> spp	Giemsa stain	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h
		Blood	EDTA tube, RT, 2 h
<i>Toxoplasma gondii</i>	<i>Toxoplasma</i> NAAT	Cerebrospinal fluid, 1 mL serum, plasma	Closed container, clot tube (blood), EDTA tube (blood), RT, 2 h
	<i>Toxoplasma</i> antibodies, IgM and IgG ^k	Cerebrospinal fluid and/or 1 mL serum	Closed container or clot tube (blood), RT, 2 h
	Giemsa stain, histology	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h

Table II-2 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Prion			
Creutzfeldt-Jakob disease ^l	14-3-3 protein	Cerebrospinal fluid	Closed container, RT, 2 h
	Neuron-specific enolase (NSE)	Cerebrospinal fluid	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, closed container, RT, 2 h

Abbreviations: CF, complement fixation; CMV, cytomegalovirus; DFA, direct fluorescent antibody; EBV, Epstein-Barr virus; HHV-6, human herpes virus 6; HSV, herpes simplex virus; IFA, indirect fluorescent antibody; IHC, immunohistochemical stain method; IIF, indirect immunofluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; LCM, lymphocytic choriomeningitis virus; NAAT, nucleic acid amplification test; RMSF, Rocky Mountain spotted fever; RT, room temperature; VCA, viral capsid antigen; VZV, varicella-zoster virus; 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) [21].

^b Sensitivity of WNV NAAT in immunocompetent host is <60% [21]. 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, St. Louis and California encephalitis viruses

^d Detection of VZV DNA in CSF (approximately 60% of cases), CSF IgM, or intrathecal antibody synthesis distinguishes meningoencephalitis from post infectious, immune-mediated process [19].

^e Quantitative EBV NAAT may help distinguish true positive from latent virus [19].

^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 [19].

^g In HIV patients, detection of CMV DNA in CSF has 82%–100% sensitivity and 86%–100% specificity for diagnosing CNS CMV infection [19].

^h Contact state public health department to arrange testing; Questions regarding sampling techniques and shipping may be directed to the Rabies section at the CDC (404)-639-1050.

ⁱ Available at the California State Department of Health Services and the Centers for Disease Control and Prevention [22].

^j Consider if eosinophilia or exposure to raccoon feces [23]. Testing available at Dept of Veterinary Pathobiology, Purdue University (West Lafayette, IN; phone 765-494-7558).

^k Refer positive IgM to *Toxoplasma* Serology Laboratory in Palo Alto, CA 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) [24].

^l Testing available at the National Prion Disease Pathology Surveillance Center (NPDPSC) <http://www.cjdsurveillance.com> [25]. The 14-3-3 protein has limited specificity for prion disease.

^m Compliance with appropriate infection control protocols is essential.

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.”
- The Gram stain is useful in the diagnosis of conjunctivitis so two 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.
- Normal skin flora are usually not involved in conjunctivitis.

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 performed using swabs (Table III-1). The most commonly collected specimens are from the conjunctiva. Cultures for aerobic bacteria and detection of *Chlamydia* and viruses either by culture or nucleic acid amplification testing (NAAT) are most commonly performed. Because 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

Table II-3. Laboratory Diagnosis of Focal Parenchymal Brain Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacterial			
Aerobes: <i>Streptococcus</i> , <i>Staphylococcus</i> , <i>Enterobacteriaceae</i> , <i>Pseudomonas</i> , <i>Haemophilus</i> , <i>Listeria</i> spp	Gram stain	Aspirate of abscess contents, tissue	Sterile anaerobic container, RT, immediately
Anaerobes: <i>Bacteroides</i> , <i>Fusobacterium</i> , <i>Prevotella</i> , <i>Actinomyces</i> , <i>Clostridium</i> , <i>Propionibacterium</i> spp	Aerobic and anaerobic bacterial culture (<i>Propionibacterium</i> culture should be held up to 14 d)		
<i>Nocardia</i> spp	Gram stain, modified acid fast stain	Aspirate of abscess contents, tissue	Sterile container, RT, immediately
	Aerobic bacterial culture (hold 7 d; add buffered charcoal yeast extract [BCYE] agar)		
	Histology (Gomori Methenamine Silver [GMS], Gram stain)	Tissue	Closed container, RT, 2 h
<i>Mycobacterium tuberculosis</i>	AFB smear	Aspirate of abscess contents (no swabs), tissue	Sterile container, RT, 2 h
	AFB culture		
	Histology (AFB stain)	Tissue	Closed container, RT, 2 h
	<i>M. tuberculosis</i> NAAT ^a	Aspirate, tissue	Closed container, RT, 2 h
Fungal			
<i>Candida</i> spp	Calcofluor stain		
<i>Cryptococcus</i> spp	Fungal culture	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
<i>Aspergillus</i> spp	Histology (GMS stain)	Tissue	Closed container, RT, 2 h
Zygomycetes (<i>Rhizopus</i> , <i>Mucor</i> sp)	Mucicarmine stain for <i>Cryptococcus</i>		
<i>Scedosporium apiospermum</i>			
<i>Trichosporon</i> spp			
<i>Trichoderma</i> spp			
Dematiaceous moulds (<i>Cladophialophora bantiana</i> , <i>Bipolaris</i> spp, <i>Exophiala</i> spp)			
Endemic dimorphic fungi			
Parasitic			
<i>Toxoplasma gondii</i>	<i>Toxoplasma</i> NAAT	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	<i>Toxoplasma</i> antibodies, IgM and IgG ^b	1 mL serum	Clot tube, RT, 2 h
	Giemsa stain	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	Histology		Formalin, indefinite
<i>Taenia solium</i> (neurocysticercosis)	<i>T. solium</i> antibodies, IgG, ELISA, confirmatory Western blot ^c	1 mL serum	Clot tube, RT, 2 h
	Histology ^d	Brain tissue	Closed container, RT, 2 h Formalin, indefinite

Table II-3 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Acanthamoeba</i> 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
	<i>Acanthamoeba</i> antibody, IFA ^e	1 mL serum	Clot tube, RT, 2 h
	<i>Acanthamoeba</i> IIF staining ^e	Brain tissue	Closed container, RT, 2 h
<i>Balamuthia mandrillaris</i>	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h Formalin, indefinite
	<i>Balamuthia</i> antibody, IFA ^e	1 mL serum	Clot tube, RT, 2 h
	<i>Balamuthia</i> IIF staining ^e	Brain tissue	Closed container, RT, 2 h

Abbreviations: AFB, acid fast bacillus; IFA, indirect fluorescent antibody; IIF, indirect immunofluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

^a A negative result does not rule out *M. tuberculosis*.

^b Refer positive IgM to Toxoplasma Serology Laboratory in Palo Alto, CA, for confirmatory testing (<http://www.pamf.org/serology/>). The absence of IgM or IgG does not exclude Toxoplasma infection [24].

^c Only 50% sensitivity if patient has solitary parenchymal lesion [24]; 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 [22].

NAAT [26]. Specimens for viral cultures should be submitted on ice, especially if specimen transport is prolonged.

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, biopsies, or anterior chamber or vitreous aspirates. 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, and 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

Table II-4. Laboratory Diagnosis of Central Nervous System Shunt Infections

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

Abbreviation: RT, room temperature.

Table II-5. Laboratory Diagnosis of Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis

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

Abbreviations: AFB, acid fast bacillus; NAAT, nucleic acid amplification test; RT, room temperature.

^a Negative NAAT for tuberculosis does not rule out *M. tuberculosis*.

presentation are used to narrow the organism(s) sought and the laboratory tests requested. Because of the limited specimen size seen with scrapings and biopsies, the laboratory and ophthalmologist may agree to inoculate these specimens onto media and prepare smears at the bedside. In this case, the laboratory should supply the necessary media and slides to the ophthalmologist. If these supplies are stored in the clinic or operating suite for ready access by the surgeon, it is the laboratory's responsibility to assure that these materials are not outdated. 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 leakproof 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.

A. 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 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* [27]. 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.

B. 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 [28]. 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 work-up. 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.

Table III-1. Laboratory Diagnosis of Periocular Structure Infections/Conjunctivitis, Orbital and Periorbital Cellulitis, Lacrimal and Eyelid Infections

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

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

^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.

C. Conjunctivitis

Most cases of conjunctivitis are caused by bacteria or viruses that are typically associated with upper respiratory tract infections [29, 30]. 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 [31]. When tests are requested, diagnosis of bacterial conjunctivitis is often compromised by the prior use of empiric antibacterial therapy [29, 30]. Sexually active patients who present with bacterial conjunctivitis should have an aggressive diagnostic work-up with Gram stain and cultures because of their risk for *Neisseria gonorrhoeae* conjunctivitis. This is a sight-threatening infection that can result in perforation of the globe. In the developing world, trachoma, a form of

conjunctivitis due to specific strains of *Chlamydia trachomatis*, is a leading cause of blindness, especially in children [32]. Off-label use of commercial NAAT assays is used for detection of this agent in research settings [33]. 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 *Propionibacterium acnes* are considered pathogens, especially in patient post-cataract or LASIK surgery [26]. 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 [26]. Most cases of neonatal conjunctivitis are due to either *Neisseria gonorrhoeae*, *Chlamydia 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*, direct fluorescent antibody testing, if available, can be used [26].

D. Keratitis

Corneal infections usually occur in 3 distinct patient populations: those with ocular trauma by foreign objects, those with postsurgical complications of corneal surgery, and in patients who practice poor hygiene associated with their extended wear contact lenses [26, 34]. Corneal infections can also result from reactivation of herpes viruses including herpes simplex virus and varicella zoster virus [35]. Post-vaccination keratitis is a well-recognized complication of vaccinia vaccination and should be considered in the appropriate clinical setting [36]. It is important to note that the use of dyes and topical anesthetics may inhibit NAAT reactions used to diagnose keratitis. The eye surface should be thoroughly rinsed with nonbacteriostatic saline before specimens for NAATs are obtained [37, 38] (Table III-2).

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 [39, 40]. *Pseudomonas aeruginosa* is the most common cause of sporadic contact lens associated keratitis but outbreaks of keratitis due to contamination of contact lens care solutions have been recently reported with both *Fusarium* and *Acanthamoeba* [39–42]. Post-surgical keratitis infections are frequently due to either coagulase-negative staphylococci and *P. acnes*, so in this setting these organisms should not be considered contaminants but as potential pathogens [26].

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, moulds including dematiaceous fungi, and environmental mycobacteria [26].

E. 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 on the mode of pathogenesis, the spectrum of causative agents will vary (Table III-3). Specimens for diagnosis of endophthalmitis can be obtained by aspiration of aqueous or vitreous fluid or via biopsy [43]. Specimen amounts are small, so discretion must be exercised in

determining for which agents the specimen should be examined. Post-operative endophthalmitis is most often caused by gram-positive organisms with coagulase-negative staphylococci predominating; chronic post-operative endophthalmitis can be due to *P. acnes*, so this organism should not be dismissed as a contaminant [44, 45]. 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 [45, 46]. Endogenous endophthalmitis, because of its association with bacteremia and fungemia, is usually caused by those organisms most responsible for bloodstream infections (eg *Candida albicans* and related species, *Aspergillus* spp, *S. aureus*, *S. pneumoniae*, the *Enterobacteriaceae*, especially *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) [45, 47, 48]. 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 [39, 49] and typically involve the uvea and retina. For further information on the diagnosis of ocular infections caused by *Onchocerca volvulus*, see Section XV-C.

F. Uveitis/Retinitis

The inflammation characteristic of uveitis/retinitis is typically due to either autoimmune conditions or is idiopathic [50]. Only infrequently is it due to infection that 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 performed preferably by an ophthalmologist with specific infectious disease expertise. Important causes of uveitis/retinitis include *Toxoplasma gondii*, cytomegalovirus, HSV, VZV, *Mycobacterium tuberculosis*, and *Treponema pallidum* [49, 51–53].

T. 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 but because the specimen needed for testing can only be obtained by a highly invasive procedure, it is unlikely that this technique will be used outside the research setting [54]. NAAT of blood, vitreous or aqueous fluids, is not as sensitive as intraocular antibody determinations, but the specimens for testing may be

Table III-2. Laboratory Diagnosis of Peri-ocular Structure Infections/Keratitis

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

Abbreviations: AFB, acid fast bacillus; BCYE, buffered charcoal yeast extract; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; RT, room temperature.

^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 buffered charcoal yeast extract, can enhance recovery of *Nocardia*.

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

^e At least 1 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 *E. coli* or some other member of the Enterobacteriaceae (ie, cocultivation) 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.

Table III-3. Laboratory Diagnosis of Endophthalmitis

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

Abbreviations: AFB, acid fast bacillus; 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 for Table 2). 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* spp 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 post-surgical 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 room temperature for inoculation into broth media and subsequent instrument-based processing.

^f Among the fungi listed, *Candida albicans*, *C. 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.

more easily obtained. Sensitivities of NAATs ranging from 50% to 80% have been reported in patients with *T. gondii* retinitis depending on the sequence used and the specimen tested. It should be noted that the total numbers of specimens tested in these studies are small, so the diagnostic value of NAAT in *T. gondii* retinitis is not yet clear [55, 56].

Since the advent of highly active antiretroviral treatment (HAART), cytomegalovirus (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 [53]. 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 [57]. Because of inter-laboratory variation in viral quantification, what represents a positive CMV viral load and a high CMV viral load will vary among laboratories [58]. Physicians should consult the laboratory performing the CMV viral load for assistance with test interpretation. As with CMV viral loads, persistent CMV antigenemia also predicts a higher likelihood of retinal disease progression and death [59].

Patients with syphilitic uveitis frequently have central nervous system findings either associated with acute syphilitic meningitis or neurosyphilis. VDRL testing of cerebrospinal fluid is recommended in clinical settings where syphilitic uveitis is suspected [51] (see section II-A).

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 [60]. Odontogenic infections are usually caused by endogenous periodontal or gingival flora. These infections include peritonsillar and pharyngeal abscesses, deep space abscesses, such as those of the retropharyngeal, parapharyngeal, submandibular, and sublingual spaces, and cervical lymphadenitis [61, 62]. 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 [63, 64]. Accurate etiologic diagnosis depends on collection of an aspirate or biopsy of inflammatory material from affected tissues and tissue spaces while

avoiding contamination with mucosal flora. 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 possibly identify mixed aerobic and anaerobic infections [65]. Additionally, spirochetes (often involved in odontogenic infection) cannot be recovered in routine anaerobic cultures but will be seen on the smear.

Infections caused by oropharyngeal flora include epiglottitis, mastoiditis, inflammation of salivary tissue, and suppurative parotitis [60, 66]. 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 [60, 67]. In addition, mycobacteria, staphylococci, and gram-negative bacilli occasionally are implicated. 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 so that the specimen remains moist. Because anaerobic bacteria are infrequent pathogens in these infections, anaerobic transport is not needed routinely. Note that fungi are common causes of chronic sinusitis, and they may not be recovered on swabs, even those obtained endoscopically. Endoscopic sinus aspirates 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 [67, 68]. Although oral flora may play an occasional etiologic role, gram-negative bacilli and staphylococci are most frequently associated with these conditions.

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.
-

Table IV-1. 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; Optimal Transport Time
Vincent Angina			
Mixed infection due to <i>Fusobacterium</i> spp and commensal <i>Borrelia</i> 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			
<i>Haemophilus influenzae</i>	Gram stain	Clinical diagnosis may not require specimen	Swab transport device, RT, 2 h
<i>Streptococcus pneumoniae</i>	Aerobic bacterial culture	Swab of epiglottis ^a only if necessary	
β -hemolytic streptococci <i>Staphylococcus aureus</i> <i>Neisseria meningitidis</i>	Blood cultures	Blood, 2–4 sets	Aerobic blood culture bottle, RT, immediately
Immunocompromised Host			
Same bacteria as in the normal host above but also other agents such as <i>Pasteurella multocida</i>	Gram stain	Clinical diagnosis may not require specimen	Swab transport device, RT, 2 h
	Aerobic bacterial culture	Swab of epiglottis ^a only if necessary	
	Blood cultures	Blood, 2–4 sets	Aerobic blood culture bottle, RT, immediately
<i>Aspergillus</i> spp	Calcofluor-KOH stain	Biopsy or protected specimen brush.	Sterile container, RT, 2 h
Other fungi	Fungal culture	Swab much less likely to recover fungi.	
	Fungal blood cultures	Blood, 2–4 sets	Aerobic blood culture bottle formulated for fungi, RT, immediately, or Lysis-centrifugation blood culture tubes, RT, immediately
Peritonsillar Abscess			
<i>Streptococcus pyogenes</i>	Gram stain	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile anaerobic container, RT, immediately
<i>Staphylococcus aureus</i>	Aerobic and anaerobic bacterial culture		
<i>Streptococcus anginosus</i> group ("S. milleri") <i>Arcanobacterium haemolyticum</i>			
Mixed aerobic and anaerobic bacterial flora of the oral cavity			
Lemierre Syndrome			
<i>Fusobacterium necrophorum</i>	Gram stain	Biopsy, aspiration or irrigation of lesion; swab not recommended	Sterile anaerobic container, RT, immediately
Occasionally mixed anaerobic bacterial flora of the oral cavity including <i>Prevotella</i> spp and anaerobic gram-positive cocci	Aerobic and anaerobic bacterial culture	Blood, 2–4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately
	Blood cultures ^b		

Table IV-1 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Submandibular, Retropharyngeal and Other Deep Space Infections Including Ludwig's Angina			
<i>Streptococcus pyogenes</i>	Gram stain	Biopsy, aspiration or irrigation of lesion; swab not recommended	Sterile anaerobic container, RT, immediately
<i>Staphylococcus aureus</i>	Aerobic and anaerobic bacterial culture		
<i>Streptococcus anginosus</i> (<i>S. milleri</i> group)	Blood cultures ^b	Blood, 2–4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately
<i>Actinomyces</i> spp			
Mixed aerobic and anaerobic bacterial flora of the oral cavity			
Cervical Lymphadenitis			
Acute Infection			
<i>Streptococcus pyogenes</i>	Gram stain	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile anaerobic container, RT, immediately
<i>Staphylococcus aureus</i>			
<i>Streptococcus anginosus</i> (<i>milleri</i>) group	Aerobic and anaerobic bacterial culture		
Mixed aerobic and anaerobic bacterial flora of the oral cavity	Blood cultures ^b	Blood, 2–4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately
Chronic Infection			
<i>Mycobacterium avium</i> complex	Acid fast smear	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, 2 h
<i>M. tuberculosis</i>	AFB culture		
Other mycobacteria			
<i>Listeria monocytogenes</i>	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, immediately
<i>Bartonella henselae</i>	<i>Bartonella</i> NAAT ^c	5 mL plasma	EDTA tube, RT, 2 h
	<i>Bartonella</i> culture ^d	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, immediately
	Histopathology (Warthin-Starry and H&E stains)	Tissue in formalin for histopathology	Container for pathology, indefinite

Abbreviations: AFB, acid fast bacillus; 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 the 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.

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 IV-1)

B. Mastoiditis and Malignant Otitis Externa Caused by Oropharyngeal and Exogenous Pathogens (Table IV-2)

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

Table IV-2. Laboratory Diagnosis of Mastoiditis and Malignant Otitis Externa Caused by Oropharyngeal and Exogenous Pathogens

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Mastoiditis			
<i>Streptococcus pneumoniae</i>	Gram stain	Middle ear fluid obtained by tympanocentesis or biopsy of mastoid tissue; swab not recommended	Sterile anaerobic container, RT, immediately
<i>Haemophilus influenzae</i>	Aerobic and anaerobic bacterial culture		
<i>Moraxella catarrhalis</i>			
<i>Streptococcus pyogenes</i>			
<i>Staphylococcus aureus</i>			
<i>Pseudomonas aeruginosa</i>			
Enterobacteriaceae			
Anaerobic bacteria			
<i>Mycobacterium tuberculosis</i>	Acid fast smear AFB culture	Biopsy of mastoid tissue	Sterile container, RT, 2 h
Malignant Otitis Externa			
<i>Pseudomonas aeruginosa</i>	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 bacillus; RT, room temperature.

nose and throat are caused by viruses (see XIV Viral Syndromes section 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 pharyngitis and should not be sought in throat cultures; nor can nasopharyngeal cultures accurately predict the etiologic agent of sinusitis.

A. Otitis Media

Otitis media is the single most frequent condition causing pediatric patients to be taken to a healthcare provider [69]. Acute otitis media with effusion (AOME) is the clinical variant of otitis media most likely to have a bacterial etiology and as a result, most likely to benefit from antimicrobial therapy (Table V-1). *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common bacterial causes of AOME, with *S. aureus*,

Streptococcus pyogenes, and *Pseudomonas aeruginosa* occurring less commonly [70]. *Alloiococcus otitidis* is also thought to cause AOME, but additional studies are needed to determine the true significance of this organism [70]. A variety of respiratory viruses are known to cause AOME; 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 AOME 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 [69, 71]. The only representative specimen is middle ear fluid obtained either by tympanocentesis or, in patients with otorrhea or myringotomy tubes, by collecting drainage on minitipped swabs directly after cleaning the ear canal. Cultures of the pharynx, nasopharynx, anterior nares, or of nasal drainage material are of no value in attempting to establish an etiologic diagnosis of bacterial AOME.

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

B. Sinusitis

The etiological agents of sinusitis vary based upon the duration of symptoms and whether it is community-acquired or of nosocomial origin (Table V-2). *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common bacterial causes of acute maxillary sinusitis. The role of respiratory viruses in sinusitis needs further studies.

Table V-1. Laboratory Diagnosis of Otitis Media

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

Abbreviation: RT, room temperature.

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

Staphylococcus aureus, gram-negative bacilli, *Streptococcus* spp, and anaerobic bacteria are associated more frequently with subacute, chronic, or nosocomial sinusitis. The role of fungi as etiologic agents is more controversial, possibly due to numerous publications that used poor sample collection methods and thus did not recover the fungal agents. In immunocompetent hosts, fungi are associated most often with chronic sinusitis [72, 73]. 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. Swabs are not recommended for collecting sinus specimens because an aspirate is much more productive of the true etiologic agent(s). Endoscopically obtained swabs can recover bacterial pathogens but rarely detect the causative fungi [74–76]. In maxillary sinusitis, antral puncture with sinus aspiration 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 potential colonization with 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 [76].

C. Pharyngitis

Pharyngitis accounts for an estimated 40 million visits by adults to medical facilities annually in the United States. The condition occurs even more often in children. 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 not sufficiently precise to permit establishing a

definitive etiologic diagnosis on clinical and epidemiologic grounds alone [77]. Consequently, the results of laboratory tests play a central role in guiding therapeutic decisions (Table V-3). Antimicrobial therapy is only warranted in patients with pharyngitis with a proven bacterial etiology [78].

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 [77]. During the past decade, rapid antigen tests for *S. pyogenes*, in particular, 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 depends on appropriate sampling technique.

There has been a general 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, 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 [79]. Laboratories accredited by the College of American Pathologists are required to back up negative rapid antigen tests with culture.

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 post-streptococcal glomerulonephritis after infection with these species have been reported. Therefore, we have included guidance for detecting Groups C and G β -hemolytic streptococci (large colony producers, since *S. 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 is often found to cause a highly suggestive scarlatina-form rash in some patients. *Neisseria gonorrhoeae* and *Corynebacterium diphtheriae*, in very specific epidemiologic settings, may also cause pharyngitis.

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 because there exists no pathogen-directed therapy for these agents. Herpes simplex

virus (HSV), human immunodeficiency virus (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.

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. *F. 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 appropriate procedures can be available. In the absence of anaerobic capability of the laboratory, this would be sent out to a reference laboratory [80–85].

Table V-2. Laboratory Diagnosis of Sinusitis

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

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

^a *Staphylococcus aureus* and *Streptococcus pyogenes* do cause acute maxillary sinusitis but only infrequently [75].

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

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

Table V-3. Laboratory Diagnosis of Pharyngitis

Etiological Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacterial			
<i>Streptococcus pyogenes</i>	Rapid direct antigen test (followed by a secondary test if negative) ^a Direct nucleic acid amplification test (NAAT) ^b Nucleic acid probe tests ^b	Dual pharyngeal swab Pharyngeal swab Pharyngeal swab	Swab transport device, RT, 2 h Swab transport device, RT, 2 h
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
<i>Arcanobacterium haemolyticum</i> ^d	Throat culture for <i>A. haemolyticum</i>	Pharyngeal swab	Swab transport device, RT, 2 h
<i>Neisseria gonorrhoeae</i> ^d	Throat culture for <i>N. gonorrhoeae</i>	Pharyngeal swab	Swab transport device, RT, 2 h
<i>Corynebacterium diphtheriae</i> ^d	Methylene blue stain <i>C. diphtheriae</i> culture	Pseudomembrane	Sterile container, RT, immediately
<i>Fusobacterium necrophorum</i>	Anaerobic incubation. A selective medium is available	Pharyngeal swab	Anaerobic swab transport, RT, 2 h
Viral			
Epstein-Barr virus (EBV)	Monospot test ^e EBV serology	5 mL serum	Clot tube, RT, 2 h
Herpes Simplex virus (HSV) [usually Type 1]	Direct detection test ^f Culture ^f HSV IgG and IgM serology ^g	Swab of pharyngeal lesion 5 mL serum 5 mL serum	Swab transport device, RT, 2 h Clot tube, RT, 2 h Clot tube, RT, 2 h
Cytomegalovirus (CMV)	CMV IgM serology	5 mL serum	Clot tube, RT, 2 h
Human immunodeficiency virus (HIV)	(see XIV Viral Syndrome)		

Abbreviations: 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 [78]. This secondary testing is usually unnecessary in adults [79]. A convenient means of facilitating this two-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 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. Nucleic acid probe tests are usually performed on enriched broth cultures, thus requiring longer turnaround times.

^c Detection of Groups 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 *S. anginosus* ("*S. milleri*") group. Check with the laboratory to determine if these are routinely looked for.

^d *Arcanobacterium haemolyticum*, *Neisseria gonorrhoeae* and *Corynebacterium diphtheriae* only cause pharyngitis in restricted epidemiologic settings. The laboratory will not routinely attempt to 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 to aid in their detection.

^e 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. 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.

^f Probable cause of pharyngitis only in immunocompromised patients. Numerous rapid tests based on detecting HSV-specific antigen (by DFA) directly in clinical material have been developed; however the nonspecific stain Tzanck test is very insensitive and 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 that is compatible with the test to be performed. Culture may be useful in immunocompromised patients.

^g The serologic test should distinguish between IgG and IgM. Depending on the age of the patient and the specific serologic assay used, in the face of a compatible illness, a single HSV-specific IgG level may be considered presumptive evidence of HSV infection. The presence of HSV-specific IgM may be considered diagnostic.

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; healthcare-associated and

Table VI-1. Laboratory Diagnosis of Bronchitis and Bronchiolitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
<i>Mycoplasma pneumoniae</i>	NAAT ^{a,b}	Throat swab ^c , nasopharyngeal (NP) swab, sputum	NP swab, aspirate or wash Suitable transport device, RT, 2 h Clot tube, RT, 2 h
	<i>Mycoplasma</i> IgG and IgM serology (enzyme immunoassay [EIA])	5 mL serum	
<i>Chlamydia pneumoniae</i>	NAAT ^{a,b}	Nasopharyngeal (NP) swab, sputum	Suitable transport device, RT, 2 h Clot tube, RT, 2 h
	<i>Chlamydia</i> IgG and IgM serology (microimmunofluorescent stain; MIF)	5 mL serum	
<i>Bordetella pertussis</i>	<i>Bordetella</i> culture and/or NAAT	Nasopharyngeal (NP) swab	Suitable transport device, RT, 2 h
Viruses			
Influenza virus	Rapid antigen detection tests ^d	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	Suitable transport device, wet ice, 2 h
Adenovirus	Virus culture		
Parainfluenza virus			
Respiratory syncytial virus	NAAT ^e	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	Suitable transport device, wet ice, 2 h
Human metapneumovirus			
Rhinovirus			
Coronavirus			
Acute Exacerbation of Chronic Bronchitis			
Bacteria			
<i>Haemophilus influenzae</i> (nontypeable)	Gram stain	Expectorated sputum	Sterile container, RT, 2 h
<i>Moraxella catarrhalis</i>	Aerobic bacterial culture		
<i>Chlamydia pneumoniae</i>	See above under Acute Bronchitis	See <i>Chlamydia</i> and <i>Mycoplasma</i> above	See above
<i>Mycoplasma pneumoniae</i>	See above under Acute Bronchitis	See <i>Chlamydia</i> and <i>Mycoplasma</i> above	See above
<i>Streptococcus pneumoniae</i>	Gram stain		
	Aerobic bacterial culture Urine Antigen ^f	First voided clean catch urine specimen	Sterile container, RT, 2 h
Viruses			
Rhinovirus	Rapid antigen detection tests ^d	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	Suitable transport device, RT, 2 h
Coronavirus	Virus culture		Note that transport on wet ice is preferable, and recommended if transport will take >2 h
Parainfluenza virus (most often PIV3)			
Influenza virus			
Respiratory syncytial virus			
Human metapneumovirus	NAAT ^e		
Adenoviruses			

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

^a There is only one FDA cleared assay available at this time. Availability is laboratory specific. Clinician should check with the laboratory for optimal specimen source, performance characteristics, and turnaround time.

^b Avoid calcium alginate swabs for nucleic acid amplification tests.

^c While approved for use with certain commercial products, throat specimens, especially swabs, are the least desirable and provide the poorest recovery.

^d Rapid antigen tests for respiratory virus detection lack sensitivity and, depending upon the product, specificity. 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.

^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 laboratory regarding availability and performance characteristics including certain limitations.

^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 [87] and adults with chronic obstructive pulmonary disease [88].

Table VI-2. Laboratory Diagnosis of Community-acquired Pneumonia

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
<i>Streptococcus pneumoniae</i>	Gram stain	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
	Culture		
	Urine antigen ^a	Urine	Sterile container, RT, 24 h; >24 h–14 d, 2–8°C
<i>Staphylococcus aureus</i>	Gram stain	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; >2–24 h, 4°C
<i>Haemophilus influenzae</i>	Culture		
Enterobacteriaceae			
<i>Pseudomonas aeruginosa</i>			
<i>Legionella</i> species	Urine antigen	Urine	Sterile container, RT, 24 h; >24 h–14 d, 2–8°C
	<i>L. pneumophila</i> serogroup 1		
	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
<i>Mycoplasma pneumoniae</i>	NAAT ^b	Throat swab, NP swab, sputum, bronchoalveolar lavage (BAL)	Transport in M4 media or other <i>Mycoplasma</i> -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
<i>Chlamydia pneumoniae</i>	NAAT ^b	NP swab, throat washings, sputum, bronchial specimens	Transport in M4 or other specialized 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	Bronchoscopy with protected specimen brush	Sterile tube with 1 mL of saline or thioglycolate; RT, 2 h; >2–24 h
	Aerobic and anaerobic culture	Pleural fluid (if available)	Sterile container RT, without transport ≤60 min; Anaerobic transport vial RT, 72 h
Mycobacteria			
<i>Mycobacterium tuberculosis</i> and Nontuberculous mycobacteria	AFB smear	Expectorated sputum; induced sputum; bronchoscopically obtained specimens	Sterile container, RT, ≤2 h; ≤24 h, 4°C
	AFB culture		
	NAAT (No FDA-cleared direct test available)		
Fungi			
<i>Histoplasma capsulatum</i>	Calcofluor-KOH or other fungal stain	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, <2 h; ≤24 h, 4°C
	Fungal culture		
	Histology	Tissue	Sterile container 4°C; Formalin container, RT, 2–14 d
	Antigen Tests	Serum, urine, pleural fluid (if available)	Clot tube, RT, 2 d; 2–14 d, 4°C
	Serum antibody (CF)	Serum	Sterile container (urine), RT 2 h; >2–72 h, 4°C
<i>Coccidioides immitis/posadasii</i>	Calcofluor-KOH or other fungal stain	Expectorated sputum; induced sputum, bronchoscopically obtained specimens	Sterile container, RT, <2 h; ≤24 h, 4°C
	Fungal culture		
	Histology	Tissue	Formalin container, RT, 2–14 d; Sterile container 2–14 d, 4°C
	Serum antibody IgM (ID, LA, EIA)	Serum	Clot tube, RT, 24 h; >24 h, 4°C
	IgG antibody (CF, EIA)		

Table VI-2 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Blastomyces dermatitidis</i>	Calcofluor -KOH or other fungal stain	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, < 2 h; ≤24 h, 4°C
	Fungal culture		
	Histology	Tissue	Sterile container 4°C, Formalin container, RT, 2–14 d
	Antigen Tests	Serum, Urine, pleural fluid (if available)	Clot tube, RT, 24 h Sterile container 4°C, 2–14 d
	Serum antibody (CF)	Serum	Clot tube, RT, 24 h; >24 h, 4°C
Viruses			
Influenza viruses A, B	Rapid antigen detection	Nasal aspirates, nasal washes, NP swabs, throat washes, throat swabs, bronchoscopically obtained samples	
	DFA	Transport in viral transport media, RT <2 h; 5 d, 4°C; >5 d, –70°C	
	Viral culture methods NAAT ^c		
Adenovirus	DFA		
	Viral culture methods NAAT ^c		
	DFA		
Parainfluenza viruses 1–4	DFA		
	Viral culture methods NAAT ^c		
	DFA		
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			
<i>Paragonimus westermani</i>	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: BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; CF, complement fixation; DFA, direct fluorescent antibody test; EIA, enzyme immunoassay; ID, immunodiffusion; KOH, potassium hydroxide; LA, latex agglutination; NAAT, nucleic acid amplification test; NP, nasopharyngeal; 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%. However, studies have reported a 21%–54% false positive rate in children with NP carriage and no evidence of pneumonia [87].

^b Currently there is one FDA approved platform (see text). Availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics and turn around time.

^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.

ventilator-associated pneumonia; infections of the pleural space; bronchopulmonary infections in patients with cystic fibrosis; and pneumonia in the immunocompromised host. The reader is referred to various IDSA practice guidelines that have been written in recent years that describe the clinical features,

diagnostic approaches, and patient management aspects of many of these syndromes.

The Key Points below summarize some important caveats when obtaining specimens for the diagnosis of respiratory infections.

Table VI-3. Laboratory Diagnosis of Healthcare-Associated Pneumonia, Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia

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

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; 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 pre-reduced 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 [87].

^c No 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 reference [93].

^e Testing from this source is not offered in all microbiology laboratories.

^f 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.

Table VI-4. Laboratory Diagnosis of Infections of the Pleural Space

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
Aerobes			
<i>Staphylococcus aureus</i>	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
<i>Streptococcus pneumoniae</i>	As above plus <i>S. pneumoniae</i> urinary antigen	Urine	Sterile container, RT, 24 h; >24 h–14 d, 2–8°C
<i>Streptococcus pyogenes</i> <i>Haemophilus influenzae</i> <i>Streptococcus anginosus</i> (<i>milleri</i>) Enteric gram-negative bacilli <i>Pseudomonas aeruginosa</i> <i>Nocardia</i>	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
<i>Legionella</i>	Gram stain—carbolfuchsin counter stain Culture on BCYE <i>Legionella</i> urinary antigen (<i>L.</i> <i>pneumophila</i> serogroup 1 only)	Pleural fluid Urine	Sterile container, RT, 2 h; 4°C, >2–24 h Sterile container, RT, <24 h; 4° C, >24 h–14 d
Anaerobes			
<i>Bacteroides fragilis</i> group <i>Prevotella</i> species <i>Fusobacterium nucleatum</i> <i>Peptostreptococcus</i> <i>Actinomyces</i> spp	Gram stain Anaerobic culture	Pleural fluid	Anaerobic transport vial, RT, 72 h; without transport RT ≤60 min
Mycobacteria			
<i>Mycobacterium tuberculosis</i>	Acid fast stain Mycobacterial Culture NAAT ^a Histology	Pleural fluid Pleural or lung biopsy Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h 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
<i>Candida</i> spp	As above plus may be evident on Gram stain	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2–24 h
<i>Aspergillus</i>	General fungal assays (ie stains, culture, serology) plus galactomannan, (1–3)-β-D- glucan ^b	BAL Serum	Sterile container, 4°C, ≤5 d; –70°C >5 d Clot tube RT, 2 d; 4°C,
<i>Histoplasma capsulatum</i>	Fungal stain—calcofluor - KOH; other fungal stains Fungal culture Histology Antigen test ^c Serum antibody (CF)	Pleural fluid Pleural biopsy Serum, urine, pleural fluid, Serum	Sterile container, RT, 2 h; 4°C, >2–24 h Sterile container, RT, 2 h; 4°C, >2–24 h; Formalin container for histology, RT 2–14 d Clot tube, RT, 2 d; 4°C, 2–14 d Sterile container (urine and fluid), RT 2 h; >2–72 h, 4°C Clot tube RT, 2 d; 4°C, 2–14 d

Table VI-4 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Coccidioides immitis</i> / <i>posadasii</i>	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 (CF, EIA)	Serum	Clot tube, RT, 2 d; 4°C, 2–14 d
<i>Blastomyces dermatitidis</i>	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, pleural fluid, serum	Sterile container, 4°C, ≤5 d; clot tube (blood)
Parasites			
<i>Paragonimus westermani</i>	Direct microscopic examination of pleural fluid and sputum for characteristic ova	Pleural fluid Sputum	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; KOH, potassium hydroxide; LA, latex agglutination; NAAT, nucleic acid amplification test; RT, room temperature.

^a No 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.

^b Performance characteristics of these tests are reviewed in reference 12 [93].

^c May cross react with other endemic mycoses.

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

- First morning sputum is always best for culture.
- Calcium alginate swabs are not acceptable for nucleic acid amplification testing.
- Most negative rapid antigen test results should be confirmed by another method.
- Blood cultures that accompany sputum specimens may occasionally be helpful, particularly in high risk community acquired pneumonia patients.
- The laboratory should be contacted for specific instructions prior to collection of specimens for fastidious pathogens such as *Bordetella pertussis*.
- 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 on invasively obtained specimens is suggested.

A. Bronchitis and Bronchiolitis

Table VI-1 lists the etiologic agents and diagnostic approaches for acute bronchitis, acute exacerbation of chronic bronchitis and bronchiolitis, 3 clinical syndromes that involve inflammation of the tracheobronchial tree [86]. Acute bronchitis is largely due to viral pathogens and is less frequently caused by *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae*.

Bordetella pertussis should be considered in an adolescent or young adult with prominent cough. Direct fluorescent antibody testing has been replaced by nucleic acid amplification tests (NAATs) in combination with culture as the recommended tests of choice for *B. pertussis* detection. Currently, there is one FDA cleared platform for *B. pertussis* detection. *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. Bronchiolitis is almost exclusively caused by viruses and *M. pneumoniae*. Several FDA-approved NAAT platforms are available for the detection of select respiratory viruses.

B. Community-Acquired Pneumonia

The diagnosis of community-acquired pneumonia 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 VI-2 lists the more common causes of community-acquired pneumonia. 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 US).

The rationale for attempting to establish an etiology is that identification of a pathogen will focus the antibiotic management

for a particular patient. In addition, identification of certain pathogens such as *Legionella* species, influenza viruses, and the agents of bioterrorism have important public health significance. Currently, IDSA/ATS practice guidelines consider diagnostic testing as optional for the patient who is not hospitalized [89]. 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. Laboratories must have a mechanism in place for screening sputum samples for acceptability (to exclude those that are heavily contaminated with oropharyngeal flora 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 BAL” using the Combicath [KOL Bio Medical Instruments, Chantilly,

Table VI-5. Laboratory Diagnosis of Pulmonary Infections in Cystic Fibrosis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i> <i>Streptococcus pneumoniae</i> Enteric bacilli <i>Pseudomonas aeruginosa</i> <i>Stenotrophomonas maltophilia</i> <i>Achromobacter</i> spp	Culture	Expectorated sputum; throat swabs ^a ; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
<i>Burkholderia cepacia</i> complex	Culture using <i>Burkholderia cepacia</i> 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 <i>Burkholderia gladioli</i> <i>Ralstonia</i> spp <i>Cupriavidus</i> spp <i>Pandorea</i> spp	Culture	Expectorated sputum; throat swabs ^a ; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
Mycobacterium spp			
<i>Mycobacterium abscessus</i> <i>Mycobacterium avium</i> complex	Mycobacteria culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Fungi			
<i>Aspergillus</i> spp <i>Scedosporium</i> spp <i>Trichosporon</i>	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; RT, room temperature.

^a Young children <8 years. of age only; often called “gag sputum.”

^b 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.

Table VI-6. Laboratory Diagnosis of Pneumonia in the Immunocompromised Host

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
See list of bacterial agents responsible for CAP and HAP above	See Table VI-3 above	See Table VI-3 above	See Table VI-3 above
Additional bacterial pathogens of interest	Gram stain		
<i>Salmonella</i> (nontyphoidal)	Culture	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
<i>Elizabethkingae meningoseptica</i>		Bronchoscopically obtained specimens	
<i>Listeria monocytogenes</i>			
<i>Nocardia</i> and other aerobic Actinomycetes	Gram stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Modified acid fast stain	Bronchoscopically obtained specimens	
	Culture (include selective BCYE or other selective media)	Lung tissue	
<i>Rhodococcus</i>	Gram stain		
	Culture		
Viruses			
Respiratory viruses	See Tables VI-2 and 3 above	See Tables VI-2 and 3 above	See Tables VI-2 and 3 above
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 viral transport media, 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	Expectorated sputum Bronchoscopically obtained specimens	Transport in viral transport media, 4°C, 5 d; –70°C >5 d
	Use with cytologic analysis and or tissue histology for interpretation NAAT ^a	Lung tissue	Formalin container, RT, 2–14 d
Mycobacterium species			
<i>M. tuberculosis</i>	Acid fast stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	AFB Culture	Bronchoscopically obtained specimens	
	NAAT (only 1 FDA-cleared test available; for smear-positive samples)	Lung tissue	
	Histology		
<i>M. avium intracellulare</i> complex	Acid fast stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
<i>M. kansasii</i>	AFB culture	Bronchoscopically obtained specimens	Formalin container, RT, 2–14 d
<i>M. xenopi</i>	Histology	Lung tissue	
<i>M. haemophilum</i>			
Rapid growers eg, <i>M. abscessus</i>			
Fungi			
<i>Pneumocystis jiroveci</i>	DFA on BAL or sputum, (not tissue)	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2 h–7 d
	NAAT ^a	Induced sputum	
	Cytologic stains (liquid samples)	Bronchoscopically obtained specimens	Formalin container, RT, 2–14 d
		Sterile container RT, 2 h; 4°C, >2–24 h	
	Tissue stains	Tissue	

Table VI-6 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Cryptococcus neoformans</i>	Calcofluor or other fungal stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum Bronchoscopically obtained specimens	
	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
<i>Aspergillus</i> spp	Calcofluor -KOH or other fungal stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum Bronchoscopically obtained specimens Tissue	
	Galactomannan (1–3)- β -D-glucan	Serum BAL ^b	Clot tube 4°C, \leq 5 d; >5 d, –70°C Sterile container for BAL RT, 2 h; 4°C, >2–24 h
<i>Fusarium</i> spp	Calcofluor -KOH; or other fungal stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum	
	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 <i>Rhizopus</i> , <i>Mucor</i> , <i>Absidia</i> spp	Calcofluor -KOH or other fungal stain	Expectorated sputum	Sterile cup or tube RT, 2 h; 4°C, >2–24 h
<i>Pseudoallescheria boydii</i>	Fungal culture	Induced sputum Bronchoscopically obtained specimens Lung tissue	
<i>Histoplasma capsulatum</i>	Calcofluor -KOH or other fungal stain	Expectorated sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum Bronchoscopically obtained specimens Lung tissue	
	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, \leq 5 d
	Serology (CF)	Serum	RT, 2 d; 4°C, 2–14 d
<i>Coccidioides immitis/posadasii</i>	Calcofluor -KOH or other fungal stain	Expectorated sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum Bronchoscopically obtained specimens Lung tissue	
	Serum antibody IgM (ID, LA, EIA) IgG antibody (CF, EIA)	Serum	Clot tube RT, 2 d; 4°C, 2–14 d

Table VI-6 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Other endemic fungi	Calcofluor -KOH or other fungal stain	Expectorated sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	Fungal culture	Induced sputum Bronchoscopically obtained specimens Lung tissue	
	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			
<i>Toxoplasma gondii</i>	Microscopy—Giemsa stain smears (tissue)	Induced sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	NAAT ^a	Bronchoscopically obtained specimens Lung tissue	
	IgM antibody detection	Serum	Formalin container, RT, 2–14 d Clot tube RT, 2 d; 4°C, 2–14 d
<i>Enterocytozoon bieneusi</i> (Microsporidiosis)	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d
	Modified trichrome stain	Induced sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	NAAT	Bronchoscopically obtained specimens	
Cryptosporidiosis	Modified acid fast stain		
	DFA		
	NAAT ^a		
<i>Strongyloides stercoralis</i>	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d
	Microscopic wet mount examination of liquid samples for larval forms	Induced sputum	Sterile container RT, 2 h; 4°C, >2–24 h
	Culture (consult laboratory for availability)	Bronchoscopically obtained specimens	
	Histologic stains	Lung tissue	Formalin container, RT, 2–14 d

Abbreviations: AFB, acid fast bacillus; BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; CAP, community acquired pneumonia; CF, complement fixation; DFA, direct fluorescent antibody test; EIA, enzyme immunoassay; HAP, healthcare associated pneumonia; ID, immunodiffusion; KOH, potassium hydroxide; LA, latex agglutination; NAAT, nucleic acid amplification test; RT, room temperature.

^a No FDA cleared test is currently available and availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics and turnaround time.

^b Not FDA cleared for this source.

VA] 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. Recently, the FDA approved the BioFire (Salt Lake City, UT) Film Array nucleic acid amplification test (NAAT) for detection of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* [90]. Some laboratories have developed their own NAAT assays. Currently, serological testing is still considered the gold standard for these agents, although this is likely to change.

Mycobacterial infections should be in the differential diagnosis of community-acquired pneumonia (CAP) that fails to

respond to therapy for the typical CAP pathogens. *Mycobacterium tuberculosis*, while 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 in patients with chronic lung disease, cystic fibrosis, and in middle-aged or elderly thin women [91].

C. Healthcare-Associated Pneumonia, Hospital-Acquired Pneumonia, and Ventilator-Associated Pneumonia

Healthcare-associated (HCAP), hospital-acquired pneumonia (HAP), and ventilator-associated (VAP) pneumonias are

Table VII-1. Laboratory Diagnosis of Esophagitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Candida</i> spp	Calcofluor-KOH stain	Esophageal brushing or biopsy	Sterile container, RT, 2 h
	Fungus culture		
	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2–14 d
Herpes simplex virus	HSV Culture	Esophageal brushing or biopsy	Viral transport device, on ice, immediately
	Direct fluorescent stain		
	Nucleic acid amplification test (NAAT)	Esophageal brushing or biopsy	Closed container, RT, 2 h
	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2–14 d
Cytomegalovirus	CMV Culture	Esophageal brushing or biopsy	Viral transport device, on ice, immediately
	Direct fluorescent stain		
	NAAT	Esophageal brushing or biopsy	Closed container, RT, 2 h
	Immunohistochemical stain	Esophageal biopsy	Formalin container, RT, 2–14 d

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

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 HCAP, HA, and VAP in the immunocompetent patient. Table VI-3 lists the organisms most commonly associated with pneumonia in the immunocompromised patient.

Two diagnostic strategies have been recommended by the American Thoracic Society and the Infectious Diseases Society of America [92]. The clinical strategy is based on the presence of a new lung infiltrate plus the presence of 2 of 3 clinical features (fever, leukocytosis or leucopenia, and purulent secretions) [92]. Determining the cause of the pneumonia relies on initial Gram stain and semiquantitative 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. The bacteriologic strategy uses quantitative cultures of lower respiratory tract secretions obtained either bronchoscopically or via endotracheal aspiration without a bronchoscope [92]. Quantities of bacterial growth above a threshold are diagnostic of pneumonia and quantities below that threshold are more consistent with colonization. The generally accepted thresholds are as follows: Endotracheal aspirates, 10^6 CFU/mL; BAL, 10^4 CFU/mL; protected specimen brush samples (PSB), 10^3 CFU/mL. These values have significance only when the samples have been obtained >72 hours before the initiation or a change of antibiotic therapy. Quantitative studies require extensive laboratory work and special procedures that smaller laboratories may not accommodate. Bronchial washes are not appropriate for routine bacterial culture.

Table VII-2. Laboratory Diagnosis of Gastritis

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

Abbreviation: RT, room temperature.

^a Gram stain and culture of properly collected and transported stool specimens has a sensitivity of 95% as does histopathological examination. Culture may not be routinely available.

^b 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 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.

Table VII-3. Laboratory Diagnosis of Gastroenteritis, Infectious and Toxin-induced Diarrhea

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
<i>Clostridium difficile</i>	Nucleic acid amplification test (NAAT)	Stool	Closed container, RT, 2 h
	Glutamate dehydrogenase (GDH) antigen with or without toxin detection followed by cytotoxin or NAAT confirmation	Stool	Closed container, RT, 2 h
<i>Salmonella</i> spp <i>Shigella</i> spp <i>Campylobacter</i> spp	Routine stool enteric pathogen culture ^a	Stool	Closed container, RT, 2 h ^b Cary-Blair transport medium, RT, 24 h
Enterohemorrhagic <i>E. coli</i> (including <i>E. coli</i> O157:H7 and other Shiga-toxin-producing <i>E. coli</i>)	Culture for <i>E. coli</i> O157:H7 ^c Shiga-toxin immunoassay NAAT for Shiga toxin genes	Stool Stool Stool	Closed container, RT, 2 h ^b Closed container, RT, 2 h ^b Closed container, RT, 2 h ^b
<i>Yersinia</i> spp <i>Vibrio</i> spp <i>Aeromonas</i> spp <i>Plesiomonas</i> spp <i>Edwardsiella tarda</i> <i>Staphylococcus aureus</i> <i>E. coli</i> Enterotoxigenic Enteroinvasive Enteropathogenic Enteroaggregative	Specialized stool cultures ^d	Stool	Closed container, RT, 2 h ^b
<i>Bacillus cereus</i> <i>Clostridium perfringens</i> <i>Staphylococcus aureus</i>	Specialized procedure for toxin detection ^e	Stool	Closed container, RT, 2 h
<i>Clostridium botulinum</i>	Mouse lethality assay ^f (Usually performed at the State Public Health Laboratory)	Stool, gastric contents, vomitus ^g	Closed container Store and transport specimens at 4°C. Do not freeze
Parasites			
<i>E. histolytica</i> <i>Blastocystis hominis</i> ^h <i>Dientamoeba fragilis</i> <i>Balantidium coli</i> <i>Giardia lamblia</i> Nematodes including: <i>Ascaris lumbricoides</i> , <i>Strongyloides stercoralis</i> ⁱ , <i>Trichuris trichiura</i> , 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 one-vial system, 2–24 h
<i>E. histolytica</i>	<i>E. histolytica</i> species specific immunoassay	Stool	
<i>Giardia lamblia</i> ⁱ	Enzyme immunoassay	Stool	
<i>Cryptosporidium</i> spp ^j	Direct fluorescent immunoassay	Stool	
Coccidia including <i>Cryptosporidium</i> ^j , <i>Cyclospora</i> , <i>Isospora</i>	Modified acid fast stain ^k performed on concentrated specimen	Stool	
Microsporidia	Modified trichrome stain ^k performed on concentrated specimen Histologic examination with EM confirmation	Stool Small bowel biopsy	Formalin container, RT, 2–14 d

Table VII-3 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Enterobius vermicularis</i>	Pinworm paddle or Scotch tape prep	Perianal area	RT, 2 h
Virus			
Calicivirus (Norovirus ^l , Sapovirus)	NAAT	Stool	Closed container, RT, 2 h
Enteric Adenovirus			
Enterovirus/ Parechovirus ^m			
Rotavirus			
Rotavirus	Enzyme immunoassay ⁿ	Stool	Closed container, RT, 2 h
Enteric Adenovirus			
Enteric Adenovirus ^o	Viral Culture	Stool	Viral transport medium, on ice, 2 h
Enterovirus/ Parechovirus ^m			
Cytomegalovirus	Histopathological examination	Biopsy	Formalin container, RT, 2–14 d
	CMV Culture	Biopsy	Sterile container, RT, immediately
Calicivirus (Norovirus ^l , Sapovirus)	Outbreak investigation performed by public health officials	Stool	Closed container, RT, 2 h

Abbreviations: CMV, cytomegalovirus; NAAT, nucleic acid amplification test; RT, room temperature.

^a A routine stool culture in most laboratories is designed to detect *Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *E. 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 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.

^e *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.

^f 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 hours hotlines are available.

^g Implicated food materials may also be examined for *C. botulinum* toxin but most hospital laboratories are not equipped for food analysis.

^h 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.

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

^j *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.

^k These stains may not be routinely available.

^l Sporadic disease has been associated with norovirus. Testing is available at public health and some reference laboratories.

^m Asymptomatic shedding is common.

ⁿ Norovirus antigen assays have limited sensitivity and specificity and are not recommended for clinical use.

^o Enteric adenoviruses may not be recovered in routine viral culture

D. Infections of the Pleural Space

The infectious causes of pleural effusions have shifted from the traditional pneumonia pathogens of *S. pneumoniae* and *S. pyogenes* to polymicrobial infections in which anaerobic bacteria play a major role. Table VI-4 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 providing that the manufacturer's guidelines are followed with respect to the volume inoculated and whether supplementation is required to enhance recovery of fastidious pathogens such as *S. pneumoniae*. If blood culture bottles are used, an additional sample should be sent to the microbiology laboratory for Gram stain and culture of nonbacterial pathogens when indicated. Fluid should be sent for cell count, pH, protein, glucose, and

Table VII-4. Laboratory Diagnosis of Proctitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Neisseria gonorrhoeae</i>	Routine aerobic culture employing media for the recovery of <i>N. gonorrhoeae</i>	Rectal swab	Swab in Amies or Stuart's transport medium, RT, 8 h
<i>Neisseria gonorrhoeae</i>	NAAT ^a	Rectal swab	Transport is manufacturer dependent
<i>Chlamydia trachomatis</i>			
<i>Chlamydia trachomatis</i>	Direct immuno-fluorescent stain	Rectal swab	Transport is manufacturer dependent
Herpes simplex virus	Viral culture	Rectal swab	Viral transport medium, RT, 2 h, wet ice if >2 h
<i>Treponema pallidum</i>	RPR or VDRL with confirmatory <i>T. pallidum</i> specific test or syphilis IgG	Serum	Clot tube, RT, 2 h

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

^a This is not an FDA-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 turn around time.

lactate dehydrogenase (LDH). These values assist with the determination of a transudative or exudative process and in the subsequent management of the syndrome. For example, the following parameters suggest the need for drainage: pH <7.28; glucose <40 mg/dL; LDH >1000 IU/L or the presence of polymorphonuclear leucocytes (PMNs) [94]. Most infections result in an exudate or 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. An elevated adenosine deaminase level in the pleural fluid (>70 IU/L) in a patient with appropriate risk factors for tuberculosis has been shown to have a high sensitivity in high prevalence regions. A level <40 IU/L excludes the diagnosis. This marker of lymphocyte differentiation 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 this assay 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 [95].

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. A limited number of organisms have been implicated in chronic infections (Table VI-5). 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*. 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 *S. maltophilia* and *Achromobacter xylosoxidans* may be acquired during a hospital or clinic visit. *Burkholderia cepacia* complex is a very important pathogen in these patients. *B. cepacia* genomovar III (*B. 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 *B. gladioli*, *Ralstonia* spp, *Cupriavidus* spp, and *Pandorea* [96].

As CF patients have survived into adulthood, opportunistic pathogens such as nontuberculous mycobacteria have been isolated with increasing frequency. 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 older than 15 years of

age who present with exacerbations, as the incidence of *Mycobacterium* species is likely underestimated due to failure to routinely assess patients for these organisms [96].

Aspergillus fumigatus is the most common fungus recovered from CF patients where 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 [96]. *Trichosporon mycotoxinivorans* is a newly recognized pathogen that has a propensity to cause disease in patients with cystic fibrosis [97]. Table VI-5 summarizes the organisms most likely to cause exacerbation of pulmonary symptoms in CF patients [91, 96, 98, 99]. While a number of environmental non-fermenting 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 VI-6 focuses on the major infectious etiologies likely to be of interest in most immunocompromised hosts [98]. 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 bronchoalveolar lavage with or without transbronchial biopsy. 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, NAAT [100]. 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, CT-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 (GI) 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 [101]. 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.
 - 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.
-

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 VII-1). Calcofluor, potassium hydroxide (KOH), or Gram stain of esophageal brushings with histopathological examination and viral culture of esophageal biopsies will establish the diagnosis in most cases.

B. Gastritis

Both invasive and noninvasive tests (Table VII-2) are available to aid in the diagnosis of *H. pylori* infection, the major infectious etiology of gastritis [102]. 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 that 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. 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

Table VIII-1. Etiologic Agents Involved in Intra-abdominal Infections

	Enterobacteriaceae	Gram-negative; Oxidase-positive Rods	Gram-negative Nonfermenters	Gram-positive Cocci	Gram-positive Rods	Anaerobes	<i>N. gonorrhoeae</i>	<i>C. trachomatis</i>	<i>Mycobacterium</i> spp	Yeast	Dimorphic Fungi	Moulds	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	
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				

Table VIII-2. Specimen Management for Intra-abdominal Infections

Condition	Diagnostic Procedure	Optimum Specimen	Transport Issues; Optimal Transport Time
Spontaneous Bacterial Peritonitis/Ascites	Aerobic and anaerobic ^a culture	10–50 mL concentrated peritoneal fluid and	RT; if >1 h, 4°C
Secondary Peritonitis; Tertiary Peritonitis	Gram stain prior to culture	Sample in blood culture bottle ^a	
Peritoneal Dialysis-associated Peritonitis	Blood culture	2–3 sets blood culture bottles	RT, do not refrigerate
	AFB stain and culture <i>Mycobacterium</i> 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	Lesion aspirate	Anaerobic transport; RT, if >1 h, 4°C
	Gram stain specimen prior to culture		
	Blood culture	2–3 sets in blood culture bottles	RT, do not refrigerate
	Cultures for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i>	Lesion aspirates <i>C. trachomatis</i> specimen may include swab of liver capsule or surrounding peritoneum	For <i>N. gonorrhoeae</i> : Amies charcoal transport, RT. For <i>C. trachomatis</i> : Chlamydia transport medium at 4°C
	NAAT for <i>N. gonorrhoeae</i> and <i>C. trachomatis</i>	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 Antigen detection for <i>Entamoeba histolytica</i>	Serum Liver aspirate	Clot tube, RT, 2 h RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory
Infections of the Biliary Tree	Aerobic and anaerobic culture	Aspirate from lesion	Anaerobic transport device; RT, if >1 h, 4°C
	Gram stain before culture		
	Blood culture	2–3 sets	RT; do not refrigerate
	AFB stain and culture	Fluid or tissue	≤1 h at RT or 4°C
	Ova and parasite 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 <i>Entamoeba histolytica</i>	Serum	RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory
Splenic Abscess	Aerobic and anaerobic culture	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4°C
	Gram stain		
	Blood culture	2–3 sets	RT; do not refrigerate
	AFB stain and culture <i>Mycobacterium</i> 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 <i>Entamoeba</i> and <i>Echinococcus</i>	Serum	RT for <30 min, then 4°C. Freeze (–20°C) if shipping to reference laboratory.

Table VIII-2 continued.

Condition	Diagnostic Procedure	Optimum Specimen	Transport Issues; Optimal Transport Time
Secondary Pancreatic Infections	Aerobic and anaerobic culture	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4°C.
	Gram stain prior to culture		
	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 bacillus; CMV, cytomegalovirus; KOH, potassium hydroxide; NAAT, nucleic acid amplification test; 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. 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.

useful to test for organism eradication after therapy. The urea breath test is performed in the clinic. The patient ingests a cocktail containing ¹³C-labeled urea and 15–30 minutes later, a breath sample is obtained and analyzed for the presence of ¹³C-labeled CO₂ as an indication of the presence of *H. pylori* in the stomach. 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

GI infections encompass a wide variety of symptoms and recognized infectious agents (Table VII-3). The appropriate diagnostic approach to diarrheal illness is determined by the patient's age, severity of disease, duration and type of illness, time of year, and geographic location. Fecal testing is indicated for severe, bloody, febrile, dysenteric, nosocomial, or persistent diarrheal illnesses. Communication with the laboratory is required to determine what organisms, methods, and screening parameters are included as part of the routine enteric pathogen culture. Most laboratories will have the ability to culture for *Salmonella*, *Shigella*, *Campylobacter*, and test for *Clostridium difficile* and Shiga toxin-producing *Escherichia coli*. Consult with the laboratory if other pathogens are suspected; special media may be required. The specimen of choice is the diarrheal stool (ie, takes the shape of the container). NAAT tests are being developed and will eventually be the first test of choice; currently only one commercial panel has received FDA clearance, although individual Shiga-toxin NAATs are available.

Stool Culture

Stool culture is indicated for detection of invasive bacterial enteric pathogens. Most laboratories employ culture techniques

to 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 State 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 [103]. Screening algorithms that limit testing to bloody stools may also miss both O157 and non-O157 isolates. 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 Center for Disease Control and Prevention for pulsed-field gel analysis for national surveillance purposes.

Multiple stool specimens are rarely indicated for detection of stool pathogens. In studies of adult patients who submitted more than 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% [104]. In pediatric patients, the first specimen detects 98% of the enteric pathogens [105]. Thus, 1 sample for children and a second for selected adult patients may be considered. Rectal swabs are less sensitive than stool specimens and are not recommended in adults but in symptomatic pediatric patients rectal swabs and stool culture are equivalent in the ability to detect fecal pathogens [106, 107].

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 on detection of botulinum toxin in serum (in patients with wound, infant, and food-borne disease), stool (in patients with infant and food-borne disease), and gastric contents/vomit (in patients with food-borne disease). Toxin detection is performed in many State Public Health Laboratories and at the Center for Disease Control and Prevention. 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 [108].

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 for the detection of *C. difficile*. 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 has been performed. 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. With the availability of NAAT assays, EIAs for toxin alone are no longer recommended as stand-alone assays.

Nucleic acid amplification assays for the detection of *C. difficile* are available and should be considered the test of choice for the diagnosis of enterocolitis due to *C. difficile*. They have reported sensitivity of 93%–100%. To reduce turnaround time and costs, some laboratories may employ an algorithm that uses a rapid screening test for glutamate dehydrogenase (GDH) antigen with or without toxin A and B detection followed by cytotoxin or NAAT confirmation where indicated. NAAT testing should be employed if GDH antigen and toxin screening results do not agree. This algorithm allows for both the rapid reporting of most negative specimens and the sensitivity of cytotoxin testing or NAAT but could result in delays in diagnosis that range from hours to days, depending on the laboratory testing platform employed [109, 110].

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. 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 [111].

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 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 [112]. 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 [113]. 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 [114, 115]. Historically, when using conventional microscopic procedures, it was recommended that 3 specimens collected over a 7–10 days 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 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.

The specimen preservative to be employed, often supplied by the laboratory, depends on the need to perform immunoassay procedures or special stains on the specimens and the manufacturer’s recommendations for specimen fixative. Polyvinyl alcohol (PVA) is the gold standard; 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 *E. histolytica* cannot be differentiated from nonpathogenic *E. dispar* using morphologic criteria, so the laboratory report may indicate *E. histolytica/dispar* [116]. Only an immunoassay or NAAT can differentiate these organisms.

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 VII-4).

VIII. INTRAABDOMINAL 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 means 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 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 VIII-1) and how best to evaluate the situation with existing techniques (Table VIII-2).

Factors to consider when collecting specimens for laboratory diagnosis of intraabdominal infections:

Key points for the laboratory diagnosis of intraabdominal 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 tests.
- The specimen of choice for an abscess is a sample of the contents plus a sample of the wall of the abscess.
- 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 these 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 [117, 118]. SBP tends to be monomicrobial

and 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 VIII-1). Alternatively, because SBP and infections of ascites fluid tend to be monomicrobial, an aerobic blood culture bottle can be inoculated with fluid (volume dependent on 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 the organism(s) present. 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 inoculate blood culture bottles at the bedside with the fluid. 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 more than 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. Further, 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 [118, 119]. There are numerous causes of secondary peritonitis including iatrogenic or accidental trauma, perforated appendix or diverticuli, typhlitis, or intra-abdominal abscess. Unlike SBP, however, secondary peritonitis tends to be polymicrobial 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). If typhlitis is suspected, *C. difficile* toxin testing, stool cultures for enteric pathogens, and blood cultures should be requested. Additionally, *C. 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 along 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 VIII-1). Because cytomegalovirus (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 since special processing or NAAT (this specimen type has no FDA-cleared commercial platform for testing) will be necessary.

Because of the polymicrobial nature of secondary peritonitis, clinicians 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, multi-drug-resistant gram-negative bacilli, VRE, etc.) to guide empiric antimicrobial therapy [117, 118, 120]. 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.

Table IX-1. Laboratory Diagnosis of Osteomyelitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Hematogenous Seeding of Bone			
<i>Staphylococcus aureus</i>	Gram stain	Bone biopsy	Sterile container, RT, immediately
<i>Salmonella</i> spp ^a	Aerobic bacterial culture		
<i>Streptococcus pneumoniae</i> ^b			
<i>Brucella</i> spp ^c			
<i>Pseudomonas</i> spp ^d			
<i>Mycobacterium tuberculosis</i> ^e	Acid fast smear AFB culture <i>M. tuberculosis</i> NAAT ^e	Bone biopsy	Sterile container, RT, 2 h
<i>Blastomyces dermatitidis</i>	Calcofluor-KOH stain	Bone biopsy	Sterile container, RT, 2 h
<i>Coccidioides immitis</i>	Fungus culture		
Extension from a Contiguous Skin or Soft Tissue Site of Infection			
<i>Staphylococcus aureus</i>	Gram stain	Bone biopsy	Sterile container, RT, immediately
Other bacteria ^f	Aerobic bacterial culture		
Mixed aerobic and anaerobic bacterial flora of the oral cavity including <i>Actinomyces</i> spp ^g	Gram stain Aerobic and anaerobic bacterial culture	Bone biopsy	Sterile anaerobic transport container RT, immediately
Mixed bacterial flora in diabetic patients with skin and soft tissue extremity infections ^h	Gram stain Aerobic and anaerobic bacterial culture	Bone biopsy	Sterile anaerobic transport container, RT, immediately
<i>Nocardia</i> spp, other aerobic actinomycetes and soil filamentous fungi in patients with mycetoma ⁱ	Gram stain Aerobic bacterial culture Silver stain Calcofluor-KOH stain Buffered charcoal yeast extract (BCYE) agar for <i>Nocardia</i> Fungus culture	Bone biopsy or sinus tract specimen (curettage or tissue biopsy) Bone biopsy or sinus tract specimen	Sterile container, RT, immediately Sterile container, RT, 2 h

Table IX-1 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Traumatic Inoculation			
<i>Staphylococcus aureus</i>	Gram stain	Bone biopsy	Sterile anaerobic transport container, RT, immediately
Enterobacteriaceae	Aerobic and anaerobic bacterial culture		
<i>Pseudomonas aeruginosa</i> ^j			
Bacterial flora of the skin			
Bacteria found in the environment ^k			
Nontuberculous mycobacteria	Acid fast smear AFB culture	Bone biopsy	Sterile container, RT, 2 h
Environmental moulds	Calcofluor-KOH stain Fungus culture	Bone biopsy or sinus tract specimen (curetted or tissue biopsy)	Sterile container, RT, 2 h

Abbreviations: AFB, acid-fast bacillus; 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 [137].

^b *Streptococcus pneumoniae* as a cause of osteomyelitis occurs most often in pediatric patients, not infrequently in the setting of spontaneous pneumococcal bacteremia [138].

^c *Brucella* spp will be recovered in standard aerobic bacterial cultures, however, it is a slow growing bacterium and as a result, the laboratory should be notified when *Brucella* is considered to be a potential cause of osteomyelitis so that cultures can be held for examination over at least a one-week period and examined only in a biological safety cabinet. Concomitant blood cultures and serology testing are recommended (not necessary to hold blood cultures beyond standard incubation).

^d Hematogenous osteomyelitis caused by *Pseudomonas aeruginosa* and other *Pseudomonas* spp occurs most often in injection drug users [139].

^e The most common site of osteomyelitis due to *M. tuberculosis* is the vertebral bodies. This organism can also seed the clavicles and in this setting represents one of the most common causes of clavicular osteomyelitis. Commercial NAATs are not FDA-cleared for nonrespiratory sites, so a laboratory-validated test method must be used if NAATs are requested.

^f Infections of skin and soft tissues, especially with extension of infection into deeper tissue spaces, pose a risk for the development of osteomyelitis of adjacent bone. While *S. aureus* is the most commonly incriminated organism, essentially any bacterium capable of causing deep soft tissue infection can also cause osteomyelitis.

^g 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* is suspected, specimens should be transported to the laboratory and then processed within 15 minutes or there is little chance of recovering *Actinomyces* in culture.

^h Diabetic extremity infections with underlying osteomyelitis can be caused by a diverse group of bacteria including *S. aureus*, Group B β -hemolytic streptococci, *Enterococcus* spp, the Enterobacteriaceae, *Pseudomonas* spp, *Stenotrophomonas maltophilia* and a variety of anaerobes. This represents one of the few settings in which osteomyelitis can be polymicrobial. Superficial debridement followed by deep sampling at the advancing margin of the lesion is essential to avoid being misled by surface colonizing contaminants.

ⁱ 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. The etiologic agents (see table) 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. Further, the laboratory should be notified of the possibility of *Nocardia* as a pathogen so that appropriate media, eg *Neisseria* selective media and *Legionella* selective agar, can be inoculated which facilitate recovery of this organism.

^j *Pseudomonas aeruginosa* is the most common bacterial cause of calcaneal osteomyelitis in individuals who develop this infection after stepping on nails while wearing sneakers.

^k Direct trauma to bone such as may occur in open fractures with contamination of the site by soil, animal feces, water, etc, may lead to the development of osteomyelitis due to essentially any microorganism present in the contamination source. This includes the Enterobacteriaceae, *Pseudomonas aeruginosa*, unusual gram-negative bacilli, *Bacillus* spp, anaerobes such as *Clostridium* spp, *Nocardia* and other aerobic actinomycetes. This represents another form of osteomyelitis that can be polymicrobial.

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 vancomycin-resistant *Enterococcus*

spp, *Candida* species, *Pseudomonas aeruginosa*, or biofilm-producing bacteria like coagulase-negative *Staphylococcus* spp. Fluid cultures from cases of tertiary peritonitis are commonly negative for bacteria [117]. In any case, cultures appropriate for spontaneous or secondary peritonitis may be helpful (Table VIII-2). 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 (PDAP)

The evaluation of dialysis fluid from patients with suspected PDAP is essentially identical to that used for SBP. Infections tend to be monomicrobial 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 [118, 121, 122]. Fungi, especially *Candida* species contribute to the same number of identified infections as anaerobes [121]. Cultures can remain negative in >20% of all cases of PDAP [121]. Again, 10–50 mL of dialysate should be collected for concentration and culture, cytospin Gram stain evaluation, analysis for protein, cell count and differential (Table VIII-2). Blood cultures are rarely positive in cases of PDAP [118]. 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 [122, 123]. 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 (*Entamoeba 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 [124–126]. In regions where *E. histolytica* disease is endemic, the use of serology or serum antigen detection tests can be helpful to exclude amebic abscess [127] whereas examination of stool for cysts and trophozoites is generally not (Table VIII-2). 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 *Streptococcus*, *S. aureus*, *Bacteroides* spp, *Fusobacterium* spp (especially with Lemierre's syndrome), *Clostridium* spp, and rarely *Candida* spp [124–126]. Aerobic and anaerobic bacterial culture should be requested (Table VIII-2). Blood cultures can also be helpful in establishing an etiology if collected prior to the institution of antimicrobial therapy [125, 126]. 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 in biliary tract infections (primarily cholecystitis and cholangitis) are the same organisms recovered from cases of pyogenic liver abscess (see above and Table VIII-1). Parasitic causes include *Ascaris* and *Clonorchis* spp or any parasite that can inhabit the biliary tree leading to obstruction [124]. 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* (*Iso-spora*) *belli*, CMV, and *Mycobacterium avium* complex [124]. 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 [128]. Infection is most likely aerobic and monomicrobial 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 [128]. 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 VIII-2). Unusual causes of splenic abscess include *Bartonella* spp, *Streptobacillus moniliformis*, *Nocardia* spp, and *Burkholderia pseudomallei* (uncommon outside of Southeast Asia or without suggestive

travel history) [129]. The laboratory should be notified if this agent is possible due to the need for increased biosafety precautions since *B. pseudomallei* is a potential bioterrorism agent. 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 [129].

H. Secondary Pancreatic Infection

Most cases of acute or chronic pancreatitis are produced by obstruction, autoimmunity or alcohol ingestion [130, 131]. Necrotic pancreatic tissue generated by one of these processes can serve as a nidus for infection [130, 131]. 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 VIII-2). 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 may arise as a consequence of hematogenous seeding of bone from a distant site, extension into bone from a contiguous soft tissue infection, extension into bone from a biofilm on a contiguous prosthesis, or direct traumatic inoculation [132]. Similarly, joint infections may develop by any of these routes, but occur most often by hematogenous seeding. From the perspective of pathophysiology, specific nature of infection and to at least some extent, clinical course, it is useful to classify bone infections based on pathogenesis. With joint infections, a classification scheme based on specific site of involvement and tempo of disease is most instructive; ie, acute versus chronic arthritis and septic bursitis.

The potential list of causative agents of bone and joint infections is diverse and is largely predicated on the pathogenesis of infection, the nature of the infection and the host [130, 134]. With few exceptions, bone and joint infections are usually monomicrobial. Rarely, such infections are polymicrobial.

Key points for the laboratory diagnosis of bone and joint infections

- Swabs are not recommended for specimen collection; aspirates or 3–6 tissue biopsies are needed to provide sufficient sample for studies
 - Concomitant blood cultures are indicated for detection of some systemic agents of osteomyelitis and joint infections, but not for prosthetic joint infection.
 - Joint fluids should have an aliquot injected into an aerobic blood culture bottle, preferably at the bedside, in addition to placing fluid in a sterile container for direct processing.
 - For prosthetic joint infection diagnosis, 3–6 separate tissue samples should be submitted. As an alternative, sonication or bead mill homogenizing of samples from the removed prosthesis are excellent methods to detect pathogens in biofilms.
 - When anaerobic bacteria are suspected, anaerobic transport containers should be used.
 - Some agents of joint infections are not culturable and require molecular methods and/or serology for detection.
-

A. Osteomyelitis

Establishing an etiologic diagnosis of osteomyelitis nearly always requires obtaining bone biopsy material for microbiologic evaluation [135]. As much specimen as possible is desirable; specimens may include pieces of intact bone, shavings, scrapings and excised necrotic material. In true osteomyelitis, the bone tissue is often so necrotic that it can be easily obtained with a curette. Swab cultures of sinus tracts are not diagnostic and are not recommended. Similarly, determining the etiology of joint infections usually requires sampling the joint space directly with aspiration of synovial fluid and/or biopsy of the synovium. Concomitant or secondary bacteremia or fungemia occurs sporadically in patients with both osteomyelitis and infections of joints, although patients with contiguous spread osteomyelitis rarely develop bacteremia and blood cultures are rarely appropriate for that population. Thus, blood cultures collected during febrile episodes are recommended for the evaluation of patients suspected of having secondary bacteremia or fungemia. Assessment of acute phase reactants or nonspecific markers of inflammation such as procalcitonin, C-reactive protein, and erythrocyte sedimentation rates are not diagnostic in patients with these infections, but they may yield helpful information during therapy. Some less common agents may require molecular detection methods, which will often need to be sent to a reference laboratory with ensuing longer turnaround time for results (Table IX-1) [136].

B. Joint Infections

In addition to spontaneous and hematogenously seeded joint infections (Table IX-2), a special category exists for prosthetic joint infections, especially infections of knee and hip prostheses, which are most often caused by coagulase-negative staphylococci [140, 141]. Laboratory diagnosis of prosthetic joint infections based on peri-surgical cultures is difficult since contamination with skin organisms is not uncommon in surgical samples. It is important to change to a fresh sterile scalpel after making the initial incision. One recommendation to differentiate true coagulase-negative staphylococcal infection from contamination occurring during surgical removal of tissue surrounding a prosthetic joint is to obtain 3–6 separate small tissue biopsies or curettings during the surgical procedure. If the same species is recovered from 3 or more of the samples, this is strong evidence of its pathogenicity [142].

Pre-surgical sampling of joint fluids from any suspected infection should be performed in the same manner as for acute arthritis (Table IX-2). A European publication documented rapid (1–2 hour) pre- and perisurgical identification of *S. aureus*, MRSA, and putative methicillin-resistant coagulase negative staphylococci from joint fluids using a rapid NAAT assay [143]. Intra-operative Gram stains have poor yield (33%–50%) but if positive, may be helpful. Shoulder joints, whether natural or prosthetic, are preferentially infected with *Propionibacterium acnes*, a normally commensal skin organism [144]. Anaerobic cultures of shoulder tissue biopsies should be incubated in anaerobic broth for up to 14 days before discarding as negative. Recent work, primarily from Mayo Clinic, recommends sonication of prosthetic joint biopsy samples and culture of the post-sonication fluid [145]. Another recent technique that was found to increase yield of bacteria and perhaps yeast from joint tissue and bone removed during prosthetic revision surgery was bead mill processing using 1 mm glass beads [146]. Since fungi and mycobacteria are extremely rare in this setting, they should not be sought without special communication with the laboratory [147, 148]. If fungi are suspected, the bead mill method would likely destroy hyphal elements, so mincing bone and tissue and direct inoculation onto fungal agar is still recommended. Both sonication and bead mill processing are not available in most laboratories.

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:

- Urine should not sit at room temperature for more than 30 minutes. Hold at refrigerator temperatures if not cultured within 30 minutes.
 - Reflexing to culture after a positive pyuria screen should be a locally approved policy.
 - Three 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.
-

IDSA guidelines for diagnosis and treatment of urinary tract infections are published [156, 139] as are ASM recommendations [158]. These provide diagnostic recommendations that are similar to those presented here (Table X-1). 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 two syndromes [159]. 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 [160]. The Gram stain is not the appropriate method to detect PMNs in urine but it can be ordered as an option for detection of high numbers of gram-negative rods when a patient is suspected of suffering from urosepsis. Because urine is so easily contaminated with commensal flora, specimens for culture of bacterial urinary tract pathogens should be collected with attention to minimizing contamination from the perineal and superficial mucosal microbiota [161]. 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 one hour to the laboratory, yield high numbers of one or more potential pathogens on culture. Interpretation of 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-

Table IX-2. Laboratory Diagnosis of Joint Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Acute Arthritis			
<i>Staphylococcus aureus</i>	Gram stain	Synovial fluid and/or synovium biopsy	Sterile container, RT, immediately
<i>Staphylococcus lugdunensis</i>	Aerobic bacterial culture		
<i>Streptococcus pyogenes</i> <i>Streptococcus pneumoniae</i>	Inoculate up to 10 mL fluid directly into aerobic blood culture bottle at bedside ^a		Aerobic blood culture bottle
Non-Group A β -hemolytic streptococci			
Enterobacteriaceae <i>Pseudomonas</i> spp <i>Kingella kingae</i> ^b <i>Neisseria gonorrhoeae</i> ^c <i>Brucella</i> spp		Brucella serology	
Parvovirus-B19 [149]	PV-B19 serology	5 mL serum	Clot tube, RT, 2 h
	PV-B19 nucleic acid amplification test (NAAT)	Synovial fluid	Closed container, RT, 2 h
Rubella [150]	Rubella serology ^d	5 mL serum	Clot tube, RT, 2 h
Chronic Arthritis			
<i>Borrelia burgdorferi</i> (Lyme Disease) [151]	Lyme serology	5 mL serum	Clot tube, RT, 2 h
	<i>B. burgdorferi</i> culture ^e	Synovial fluid	Sterile container, RT, immediately
	<i>B. burgdorferi</i> NAAT	Synovial fluid	Closed container, RT, 2 h
<i>Mycobacterium tuberculosis</i>	Acid fast smear	Synovial fluid and/or synovium biopsy ^f	Sterile container, RT, 2 h
Non-tuberculous mycobacteria	AFB culture		
<i>Candida</i> spp <i>Cryptococcus neoformans</i> <i>Blastomyces dermatitidis</i> <i>Coccidioides immitis</i> <i>Aspergillus</i> spp	Calcofluor-KOH stain Fungal culture	Synovial fluid and/or synovium biopsy	Sterile container, RT, 2 h
Septic Bursitis			
<i>Staphylococcus aureus</i> <i>Streptococcus pyogenes</i> Other streptococci Enterobacteriaceae <i>Pseudomonas</i> spp	Gram stain Aerobic bacterial culture	Bursa fluid	Sterile container, RT, immediately Inoculate up to 10 mL fluid into aerobic blood culture bottle (in addition to separate tube of fluid).
Prosthetic Joint Infections			
<i>Staphylococcus aureus</i> Coagulase negative staphylococci <i>Enterococcus</i> spp <i>Streptococcus</i> spp (group A, B and other β hemolytic types) Enterobacteriaceae <i>Pseudomonas aeruginosa</i> <i>Corynebacterium</i> spp	Aerobic bacterial culture Gram stain not useful	Multiple tissue biopsy samples. Synovial fluid Or submit the removed prosthesis in sterile container for sonication protocol.	Sterile container, RT, 2 h

Table IX-2 continued.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Propionibacterium acnes</i> Other anaerobes	Aerobic and anaerobic bacterial culture (incubate anaerobic cultures up to 14 d)		Sterile anaerobic transport container, RT, immediately.
Polymicrobial infections	Also consider submitting prosthesis (if removed) for sonication		

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

^a When sufficient synovial fluid specimen has been obtained, up to 10 mL should be transferred aseptically into an aerobic blood culture bottle and processed in a manner similar to routine blood cultures [154, 155]. This practice, however, does not obviate the value of direct specimen Gram stains and direct solid agar culture of synovial fluid specimens. These procedures should always be done in addition to inoculation of a blood culture bottle with up to 10 mL of the fluid. Dilution of active PMNs and other factors in the blood culture broth may allow recovery of the organism when direct culture yields no growth.

^b *Kingella kingae* is most often observed as a cause of septic joint infections in children and usually involves the knee [138, 152].

^c *Neisseria gonorrhoeae* may yield aberrant morphologic forms on Gram stain of synovial fluid in patients with joint infections due to this organism. Synovial fluid and synovium biopsy specimens should be processed expeditiously for culture and even then, cultures are often negative [153].

^d In a patient with a compatible illness, especially with a history of recent vaccination with the live attenuated rubella virus vaccine, a negative serologic test for rubella may be considered suggestive evidence for joint infection due to rubella virus [150].

^e Culture of synovial fluid for *B. burgdorferi* requires use of specialized media and even with expeditious processing of specimens in an experienced laboratory, rarely results in recovery of the organism. Most laboratories will need to send the sample to a reference laboratory, further delaying and compromising possible cultures. Culture is rarely done except in research settings.

^f Detection of *M. tuberculosis* or other *Mycobacterium* species by microscopy or in culture is very uncommon from synovial fluid specimens in patients with joint infections due to these organisms [148]. Synovium tissue enhances the likelihood of detection.

collected straight-catheterized sample from the same patient, a rectal-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 should be taken from the sampling port of a newly inserted device. Cultures of Foley catheter tips are of no clinical value and will be rejected. Collection of specimens from urinary diversions such as ileal loops is also discouraged because of the propensity of these locations to be chronically colonized. Chronic nephrostomy collections and bagged urine collections are also of questionable value. Multiple organisms or coagulase-negative staphylococci may be recovered in patients with urinary stents, and may be pathogenic. It is important that Urologists and Nephrologists who care for patients with complicated infections discuss any special needs or requests with the microbiology director or supervisor. Specimens from these patients may contain a mixed flora and if specific interpretive criteria are documented for these specimen types, the laboratory must be aware of the documentation and the special interpretive standards. Laboratories routinely provide antimicrobial susceptibility tests on potential pathogens in significant numbers. Specimens obtained by more invasive means, such as cystoscopy 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 less than 1000 colony forming units (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.

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-voided morning specimens of >20 mL, and requires a specific request to the laboratory so that appropriate processing and media are employed. Recovery of adenovirus in cases of cystitis requires a specific request for viral culture. A nucleic acid amplification test (NAAT) may also be available at reference laboratories for detection of adenovirus. 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 reference laboratories.

Acute bacterial prostatitis is defined by clinical signs and physical findings combined with positive urine or prostate

secretion cultures yielding usual urinary tract pathogens [162–164]. The diagnosis of chronic prostatitis is much more problematic, and the percentage of cases in which a positive culture is obtained is much lower [165]. The traditional Meares-Stamey four glass specimen obtained by collecting the first 10 mL void, a mid-stream specimen, expressed prostate secretions (EPS) and a 10 mL post-prostate-massage urine is positive if there is a ten fold higher bacterial count in the EPS than the mid-stream urine. A two-specimen variant, involving only the mid-stream 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 X-2 summarizes the approach to laboratory diagnosis of prostatitis.

Epididymitis in men under 35 years of age is most frequently associated with the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. NAATs are the most sensitive and rapid diagnostic procedures for these agents and each commercially available system has its own collection kit. Culture of *N. gonorrhoeae* is recommended when antibiotic resistance is a concern, and special media are required for antimicrobial

susceptibility testing (AST), which may be referred to a public health laboratory. In men over 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 AST 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. Systemic fungal diseases can involve the epididymis or testis, including blastomycosis, histoplasmosis and coccidioidomycosis. *Mycobacterium tuberculosis* may also involve these sites [166]. Table X-3 summarizes the approaches to specimen management for cases of epididymitis and orchitis.

Table X-1. Laboratory Diagnosis of Cystitis and Pyelonephritis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Gram-Negative Bacteria			
Enterobacteriaceae: Includes <i>Escherichia coli</i> , <i>Klebsiella</i> spp, <i>Proteus</i> spp, others <i>Pseudomonas</i> spp, other nonfermenting gram- negative rods	Routine aerobic culture Gram stain (optional, low sensitivity)	Mid-stream, clean catch or straight cath urine	Closed sterile leakproof container; refrigerate (4°C) or use urine transport tube unless delivery to laboratory ≤1 h is certain.
Gram-Positive Bacteria			
<i>Enterococcus</i> spp <i>Staphylococcus aureus</i> <i>Staphylococcus</i> <i>saprophyticus</i> <i>Corynebacterium</i> <i>ureolyticum</i> <i>Streptococcus agalactiae</i> (Group B streptococci)	Routine aerobic culture Gram stain (optional, low sensitivity)	Midstream, clean catch, or straight cath urine	Closed sterile leakproof container; refrigerate (4°C) or use urine transport tube unless delivery to laboratory ≤1 h is certain.
Mycobacteria			
<i>Mycobacterium</i> <i>tuberculosis</i>	Mycobacterial culture	First void urine	Prefer >20 mL urine, refrigerate (4°C) during transport
Virus			
Adenovirus	Virus Culture NAAT ^a	Midstream or clean catch urine	Closed sterile container to laboratory 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: NAAT, nucleic acid amplification test; RT, room temperature.

^a No FDA-cleared NAAT tests available

Table X-2. Laboratory Diagnosis of Prostatitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Acute Bacterial Prostatitis			
<i>E. coli</i> , other enteric bacteria, <i>Pseudomonas</i> spp	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
<i>Staphylococcus aureus</i>			
<i>Enterococcus</i>			
Group B streptococci			
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			
<i>Blastomyces dermatitidis</i>	Fungal culture	Expressed prostate secretions, prostate biopsy	Closed sterile container to laboratory within 1 h or refrigerate (4°C) if delayed transport
<i>Coccidioides immitis</i>			
<i>Histoplasma capsulatum</i>			
Mycobacteria			
<i>Mycobacterium tuberculosis</i>	Mycobacterial culture	First void urine, expressed prostate secretions, prostate biopsy	Prefer >20 mL urine, refrigerate (4°C) during transport

Abbreviation: RT, room temperature.

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 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, specific diagnosis has the benefit of increasing therapeutic compliance by the patient and the patient is more likely to comply with partner notification [167]. 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 re-infected by their sexual partner [168, 169]. Thus referral for partners for specific testing and treatment is essential to

prevent re-infection and is especially true for patients who may be pregnant. Finally, because the vast majority of genital infections are STIs and communicable, they are a public health concern and patients and providers should note that positive tests for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), syphilis, chancroid, and human immunodeficiency virus (HIV) require reporting in accordance with state and local statutory requirements by the laboratory and/or the provider. Reporting of additional STIs varies by state [167].

Key points for the laboratory diagnosis of genital infections:

- For vaginosis (altered vaginal flora) a Gram stain is more specific than culture or probe testing and culture is not recommended.
- Distinguishing between HSV-1 and HSV-2 antibodies requires testing with type-specific glycoprotein G (gG)-based assays.
- Testing simultaneously for CT, GC and *Trichomonas* is optimal for detection of the most common treatable STIs in female patients.
- Screen for Group B streptococcus at 35–37 weeks of pregnancy using both vaginal and rectal swabs.
- Screen for HIV early in each new pregnancy and in sexually active patients age 13–64 seeking evaluation for STIs.
- Undertake partner testing and/or treatment of positive index cases to prevent re-infection.

Table X-3. Laboratory Diagnosis of Epididymitis and Orchitis

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

A. Genital Lesions

Genital lesions may have multiple simultaneous infectious etiologies that make them a challenge to diagnose and treat properly. Centers for Disease Control and Prevention (CDC) guidelines 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 *H. ducreyi* where chancroid is prevalent. Because many of the genital lesions exhibit inflammatory epithelium that enhances the transmission of HIV, screening with an EIA (enzyme-immunoassay) HIV antibody test is recommended in these patients as well [167]. Table XI-1 shows the diagnostic tests for identifying the etiology of the most common genital lesions.

For suspected cases of HSV genital lesions, viral culture, direct fluorescent antibody (DFA) and/or nucleic acid amplification tests (NAATs) are commonly used for diagnosis. Since methods for specific testing for vesicles varies among laboratories, consultation with the laboratory before specimen collection is appropriate. For instance, while NAATs are the most sensitive, especially where suboptimal collection, or nonulcerative or vesicular lesions may be present, there may be limitations as to specimen source acceptable and patient age depending on the NAAT used. Culture is more likely to be positive in patients that have vesicular versus ulcerative lesions, specimens obtained from a first episodic lesion versus 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 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 (gG) –based assay is requested [167, 168].

Point of care tests (POCT) for HSV-2 may yield false positive results in patient populations with a low likelihood of HSV infection or in early stages of infection as well as false negative results in primary lesions that are due to HSV-1.

In children presenting with genital lesions, providers should not assume HSV only but should consider potential atypical presentation of Varicella zoster virus (VZV). DFA is best for detection of VZV as culture is less sensitive. Pregnant patients with a history of genital herpes should be assessed for active lesions at the time of delivery.

New consensus guidelines for the management of women with abnormal cervical cytologic lesions and human papilloma virus (HPV) as well as the use of genotyping tests are pending publication. The 2006 consensus guidelines are discussed in the American Journal of Obstetrics and Gynecology by Wright et al. regarding routine high-risk HPV testing and available at the website www.asccp.org/consensus/histological.shtml

(accessed 2-3-13) [170]. A more recent American Society for Colposcopy and Cervical Pathology (ASCCP) HPV genotyping update discusses the testing specifically for HPV 16/18 genotype in women over 30. Basically, HPV testing is recommended for the purposes of triaging women >20 years of age with atypical squamous cells of undetermined significance (ASC-US) or ASC-H (atypical squamous cells cannot exclude high grade squamous intraepithelial lesion [HSIL]). Only testing for those high-risk HPV types that are associated with cervical cancer is appropriate. Follow-up testing for abnormal cytology and/or positive HPV is complicated and readers are referred to the ASCCP guidelines for management decisions. In addition, recommendations for testing for genotypes 16/18 HPV for women over 30, where high risk HPV testing can be ordered in conjunction with cytology, should be considered. In general, results of cytology negative but HPV high risk positive warrant HPV 16/18 genotype determination for identification of patients at higher risk for progression to invasive cervical cancer [171]. Endocervical specimens in liquid cytology medium have a higher sensitivity for detecting significant lesions (eg squamous intraepithelial lesions (SIL) and can facilitate subsequent HPV testing in patients since it can be done from the same specimen. Patients with a cervix remaining after hysterectomy, HIV positive patients, and patients that have received the quadrivalent recombinant HPV vaccine (Gardasil from Merck and Co.) should undergo routine Papanicolaou (Pap) and HPV screening and management. Pap and/or HPV testing should be postponed when a woman is menstruating [170, 172, 173].

In the United States, testing for syphilis traditionally has consisted of initial screening with an inexpensive nontreponemal test (rapid plasma reagin, RPR), then retesting reactive specimens with a more specific, and more expensive, treponemal test (eg *T. pallidum* particle agglutination [TP-PA]). 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. However, for economic reasons, some high-volume clinical laboratories have begun using automated treponemal tests, such as automated EIAs or immunochemoluminescence tests, and have reversed the testing sequence: first screening with a treponemal test and then retesting reactive results with a nontreponemal test. This approach has introduced complexities in test interpretation that did not exist with the traditional sequence [174]. Specifically, screening with a treponemal test sometimes identifies persons who are reactive to the treponemal test but nonreactive to the nontreponemal test. No formal recommendations exist regarding how such results derived from this new testing sequence should be interpreted, or how patients with such results should be managed. To begin an assessment of how clinical laboratories are addressing this

concern, CDC reviewed the testing algorithms used and the test interpretations provided in four laboratories in New York City [174]. Substantial variation was found in the testing strategies used, which might lead to confusion about appropriate patient management. A total of 3664 (3%) of 116 822 specimens had test results (ie, reactive treponemal test result and nonreactive nontreponemal test result) that would not have been identified by the traditional testing algorithms, which obviate additional testing if the nontreponemal test result is nonreactive. If they have not been previously treated, patients with reactive results from treponemal tests and nonreactive results from nontreponemal tests should be treated for late latent syphilis.

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 *Haemophilus ducreyi*, is the one genital lesion where a Gram stain may be helpful in diagnosis. Communication with the laboratory about the presumed diagnosis and specimen transport may enhance recognition of organisms in the Gram stain and facilitate the appropriate culture technique. Samples must be obtained after surface debridement and should be sent to a referral laboratory familiar with this testing as many microbiologists have rarely seen chancroid. Lymphogranuloma venereum (LGV) is caused by the intracellular pathogen *Chlamydia trachomatis* (CT), specifically serovars L1, L2a, L2b and L3. LGV is a diagnosis of exclusion of more common entities in context with epidemiological information [168].

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 nature. However, the mode of transmission and/or acquisition is not necessarily that of an STI for BV or VVC, but it is for TV. A number of point-of-care tests (POCTs) can be performed from a vaginal discharge specimen while the patient is in the healthcare setting. Although POCTs are popular, the sensitivity and specificity of POCTs for making a specific diagnosis varies 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's diagnostic criteria) is equal to a scored Gram stain of vaginal discharge. However, a scored Gram stain is more specific than probe hybridization and POCT tests (that only detect the presence of *G. vaginalis* as the hallmark organisms for altered vaginal flora). 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 exam of vaginal specimens and interpretation are difficult for many laboratories to perform and wide variation of sensitivities (40%–70%) for both TV and CVV using smear exam exists relative to NAAT and culture, respectively. It should be noted that recent publications utilizing NAATs highlight the prevalence of *Trichomonas* as equal to or greater than CT and GC and point to a growing trend toward screening for TV, CT and GC simultaneously. Tests for the entities of vaginosis/vaginitis are shown in Table XI-2 [173, 175–178].

C. Urethritis/Cervicitis

Urethritis and cervicitis share common signs and symptoms and infectious etiologies in male and female patients, respectively. Table XI-3 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 U.S. Preventive Services Task Force [163, 179, 180].

Annual CT screening

- Sexually active women age ≤ 25 years and those pregnant
- Older women with new sex partner or multiple sex partners
- Women who are incarcerated

GC Screening (consider local epidemiology and risk)

- Sexually active women age ≤ 25 years and those pregnant
- Women with previous GC infection or other STIs
- Women experiencing multiple sex partners
- Women who do not use condoms
- Commercial sex workers and those who use drugs
- Women who are incarcerated

For laboratory diagnosis of CT and GC, many methods exist but nucleic amplification tests (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 [181]. Specifically, EIA tests for CT should not be used due to lack of sensitivity. In general, retesting patients with a follow-up test for CT or GC (test of cure) is not recommended unless special

Table XI-1. Laboratory Diagnosis of Genital Lesions

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimen	Transport Issues; Optimal Transport Time
Herpes simplex virus 1 and 2	Direct fluorescent antibody (DFA)	Scraping of lesion base rolled directly onto slide ^a	RT
Note: in children with genital lesions, consider atypical VZV ^a	Culture	Scraping of lesion base and placed in VTM/UTM ^b	RT, if >2 h, refrigerated or on ice
	NAAT ^c	Scraping or aspirate	Assay-specific; consult laboratory
	Serology ^d	Serum	Clot tube, RT
Human papilloma virus (HPV) 16/18 genotyping	DNA hybridization probe or NAAT for high-risk HPV types ^e	Endocervical brush into liquid cytology medium or transport tube	RT, 48 h
Genital warts ^f	Histopathology; HR HPV testing not done on warts	Biopsy or scraping	Formalin container, RT, 2–24 h
Syphilis	Darkfield microscopy ^g	Cleanse lesion with gauze and sterile saline	RT, immediately to laboratory
	Test is not widely available and specimen must be transported to laboratory immediately to visualize motile spirochetes	Swab of lesion base directly to slide	
	DFA- <i>Treponema pallidum</i> (DFA-TP) ^{h,i}	Cleanse lesion with gauze and saline	Slide should be dry before placing in holder and/or transporting to lab
		Swab of lesion base directly to slide	
	Serology	Serum	Clot tube, RT, 2 h
	Non-Treponemal (VDRL or RPR) ^j		
	Treponemal Serology	Serum	Clot tube, RT, 2 h
	EIA or TP-PA, FTA-ABS ^{k,l}		

Table XI-1 continued.

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimen	Transport Issues; Optimal Transport Time
Chancroid (<i>Haemophilus ducreyi</i>)	Gram stain and Culture ^m NAAT ^c	Swab of lesion base without surface genital skin	RT immediately to laboratory
Lymphogranuloma venereum (LGV)	Cell culture ⁿ	Swab of ulcer base, bubo drainage, rectum	RT, immediately to laboratory
<i>Chlamydia</i> serovars L1, L2, L2a, L2b, L3)	Serology	Serum	RT, 2 h
	Microimmunofluorescence (MIF) ^o		
	Serology	Serum	RT, 2 h
	Complement fixation (CF) ^p NAAT ^q	Swab of ulcer base, bubo drainage, rectum	RT, 2 d; or refrigerate
Granuloma inguinale (donovanosis) <i>Klebsiella granulomatis</i>	Giemsa or Wright stain in pathology. Visualization of blue rods with prominent polar granules	Scraping of lesion base into formalin	RT, 2 h
Scabies/lice	Macro and microscopic visualization	Collect parasite from skin scrapings onto slide; place in a sterile Petri dish ^f	RT, within 1 h

Abbreviations: EIA, enzyme immunoassay; NAAT, nucleic acid amplification test; RT, room temperature; VZV, Varicella zoster virus.

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

^b VTM – viral transport medium or UTM – universal transport medium. Check with laboratory, some types can be maintained and shipped at RT.

^c NAAT – nucleic acid amplification test; several NAATs are FDA-cleared. Specimen source and test availability are laboratory specific. Provider needs to check with laboratory for allowable specimen source and TAT. More sensitive than culture or DFA when lesions are past vesicular stage.

^d Serology can be nonspecific for HSV-1 and HSV-2 differentiation; should be limited to patients with clinical presentation consistent with HSV but with negative cultures; for determination of asymptomatic carriers; request type-specific glycoprotein G (gG)-based assays that differentiate HSV-1 and HSV-2.

^e High-risk (HR) HPV testing currently only recommended in women with Pap smear showing atypical squamous cells of undetermined significance (ASC-US) or >30 years of age. HPV testing is not recommended for the diagnosis of HPV in a sexual partner or in patients <20 y/o (adolescents) with ASC-US. HPV 16/18 genotyping in cytology negative and HR HPV positive specific guidelines pending.

^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 DFA-TPA - Limited availability, typically performed in public health laboratories.

ⁱ Viable organisms are not required for optimal test performance.

^j Non-treponemal tests – (rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL); less sensitive in early and late disease; become negative after treatment; do not use to test pregnant patients due to potential for false-positive results.

^k Treponemal tests – Enzyme immunoassay (EIA) formats, *T. pallidum* particle agglutination (TP-PA) and fluorescent treponemal antibody absorbed (FTA-ABS); monitor titers using same type of test and/or same lab; positive for life; HIV positive patients may have unusual serologic responses.

^l EIA – treponemal enzyme immunoassay test may be performed first with subsequent testing done with non-treponemal test such as RPR (reverse testing algorithm). Confirmation with a TP-PA test may be required in positive EIA but negative RPR.

^m Gram stain with chancroid organisms shows small rods or chains in parallel rows, “school of fish”; culture requires special media and sensitivity only 30%–70%. Consider sending slide and culture to a referral laboratory familiar with this testing.

ⁿ Cell culture sensitivity about 30%; rectal ulcers in MSM.

^o MIF titers ≥ 256 with appropriate clinical presentation suggests LGV.

^p CF titers ≥ 64 with appropriate clinical presentation suggests LGV, sensitivity 80% at 2 weeks.

^q NAATs for CT will detect L1-L3 but do not distinguish these from the other CT serovars.

^r Place a drop of mineral oil on a sterile scalpel blade. Allow some of the oil to flow onto the papule. Scrape vigorously six or seven 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.

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 re-infection 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. Appropriate providers or laboratories that perform testing in children should be consulted [167]. NAATs on samples other than genital are currently not FDA-cleared and require in-house laboratory validation.

Recently, prevalence studies using NAATs have shown that *Trichomonas* is as common as CT and more common than GC

Table XI-2. Laboratory Diagnosis of Bacterial Vaginosis, Yeast Vaginitis, and Trichomoniasis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Yeast (pH <4.5 ^a)	Saline wet mount and 10% KOH ^b _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	RT, 7 d
Bacterial vaginosis (BV) (pH >4.5 ^a)	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	RT, 7 d
Trichomoniasis (pH >4.5 ^a)	Saline wet mount ⁱ	Swab of vaginal discharge	Submitted in saline, RT, 30 min (optimal) – 2 h
	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
	NAAT ^l	Vaginal, endocervical swab, urine or liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	RT, 7 d (or manufacturer's recommendation)

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

^a pH of vaginal discharge for each condition listed when using pH strips as a point of care test.

^b KOH – potassium hydroxide.

^c Sensitivity of wet mount between 40% and 80%.

^d Culturette (BD Microbiology Systems, Sparks, Md) or similar product.

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

^f Affirm VP III Assay (Becton Dickinson, Sparks, Md); does not rely on viable organisms for optimal test performance; special transport tube required; detects *G. vaginalis* as an organism associated with BV, yeast vaginitis (*C. albicans* only) and *Trichomonas vaginalis*. 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 BV; culture not recommended; testing and treatment recommended in symptomatic pregnant patients to reduce postpartum endometritis [8].

ⁱ Wet mount for trichomonads requires live organisms to visualize movement and has poor sensitivity.

^j OSOM Trichomonas Rapid Test (Genzyme, Diagnostics, Cambridge, MA); 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, OR) allows both immediate smear review by wet mount and subsequent culture; not widely available, sensitivity approximately 70% compared to NAAT methods.

^l NAAT- nucleic acid amplification test; APTIMA Trichomonas vaginalis (ATV) test (Gen-Probe, Inc. San Diego, CA) is a recently FDA-cleared test for both screening as well as diagnosis of TV in women. Multiple specimen types can be used. Same specimen and collection device as currently used for Aptima CT/GC NAAT. Testing for males and alternate sites has been validated by some laboratories. Provider needs to check with laboratory for availability. Some laboratories have validated an in-house PCR method. Check laboratory for availability and specimen types allowed.

in most clinical and geographic settings, with a uniquely high presence in women over 40 and incarcerated populations. In addition, the ulcerative nature of the 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. An FDA-cleared NAAT allows testing from the same screening specimens used for CT and GC testing with significantly improved sensitivity over wet mount.

Standardized tests for *M. genitalium* are not available or recommended. However, in patients with nonchlamydial, NGU (nongonococcal) urethritis, 15%–25% infections may be due to

this organism. A NAAT may be the best option for detection of *M. genitalium*, due to issues with culture and cross-reactivity with serologic tests, but that test is neither FDA-cleared nor widely available. Culture for *Ureaplasma* is not recommended because of the high prevalence of colonization in asymptomatic, sexually active people [182, 183].

D. Infections of the Female Pelvis

Pelvic inflammatory disease (PID) is a spectrum of disorders of the upper female genital tract and includes any single or combination of endometritis, tubo-ovarian abscess, and salpingitis.

PID can be clinically difficult to identify when patients present with mild or nonspecific symptoms. Finding symptoms on physical exam (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 nonaseptically collected specimens (endocervical or dilatation and curettage [D and C] have limited utility in diagnosing PID. *Actinomyces* spp can cause infections associated with intrauterine devices; if suspected, the laboratory should be notified to culture such samples anaerobically, including an anaerobic broth that is held for 7 days. Patients with suspected PID should be tested for CT, GC, TV and HIV. Both difficulty in diagnosis as well as significant potential sequelae should make the threshold for therapy low [184, 185].

Postpartum endometritis should be suspected when the patient presents with high fever ($\geq 101^{\circ}\text{F}$ or $>100.4^{\circ}\text{F}$ (38.0°C) on more than two occasions >6 hours apart after the first 24 hours of delivery and up to 10 days post delivery.) after the first 24 hours post-delivery, abdominal pain, uterine tenderness and foul lochia. Usually a multi-organism syndrome, the infection is most commonly seen in patients with unplanned cesarean section 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. Screening for colonization with group B streptococci (both vaginal and anal swabs) at 35–37 weeks gestation and prophylaxis during labor and delivery can reduce the incidence of neonatal disease [186]. 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 XI-4.

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 Jenny, Kellogg, Girardet, and Black where NAAT and noninvasive specimens have yielded excellent results [187–189].

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, pharynx and urethra. Readers are referred to the CDC Treatment Guidelines for further recommendations [167].

In pregnant patients, screening for HIV, syphilis, hepatitis B surface antigen (HBsAg), CT, 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 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 both *Trichomonas* and Group B streptococci. While NAATs are available for Group B streptococci, the sensitivity is optimal only when performed from an enrichment broth specimen. Group A streptococci are not detected by Group B 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, eg 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 [190]. Screening tests (serology, stool cultures) in pregnant women are not appropriate.

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, 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 pre-existing 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 (DFI) typically originate in a wound, secondary to a neuropathic ulceration. Anaerobic bacteria are important and predominant pathogens in DFI 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* species), 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 [191].

Table XI-3. Laboratory Diagnosis of Pathogens Associated with Cervicitis/Urethritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
<i>Chlamydia trachomatis</i> (CT)	NAAT ^a	Urine Endocervical, vaginal and/or urethral swab (rectum, pharynx, conjunctiva, liquid-based cytology) ^{b,c}	Laboratory-provided transport device, RT, 2 d
	Hybridization probe ^{d,e}	Endocervical or urethral swab ^d	Laboratory-provided transport device, RT, 2 d
	Culture ^{e,f}	Endocervical, urethral, conjunctival, nasopharyngeal (NP), pharynx, or rectal swab	Laboratory-provided transport device, Refrigerate (4°C); <2 h
	Direct fluorescent antibody (DFA) test ^g	Conjunctival swab	Transport medium, RT, 2 h
<i>Neisseria gonorrhoeae</i>	Gram stain ^h	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
	Hybridization probe ^d	Endocervical or urethral swab	Laboratory-provided transport device, RT, 2 d
	Culture ⁱ	Endocervical, urethral, conjunctival, nasopharyngeal, pharynx, rectal swab	Transport medium, RT, ≤1 h Do not refrigerate specimen
<i>Trichomonas vaginalis</i>	Saline wet mount ^j	Endocervical or urethral swab	Submit in 0.5 mL saline, 30 min–2 h
	Rapid antigen test ^k	Endocervical swab	Laboratory-provided transport device, RT, 24 h
	DNA hybridization probe ^l	Endocervical or vaginal swab	Laboratory-provided transport device, RT, 7 d
	Culture ^m	Endocervical or urethral swab	Direct inoculation into InPouch TV culture system, 2–5 d ⁿ
	NAAT ^c	Vaginal, endocervical swab, urine and liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	Laboratory-provided transport device, RT, 2 d

Table XI-3 continued.

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Herpes simplex virus	DFA ^a	Scraping of lesion base	Apply to slide at bedside, RT, 24 h
	Culture	Scraping of lesion base	Place in VTM/UTM, RT or on ice, 2 h
	NAAT ^c	Scraping of lesion or swab of discharge	Laboratory-provided transport device, Assay-specific; consult laboratory ⁿ

Abbreviations: NAAT, nucleic acid amplification test; RT, room temperature; UTM, universal transport media; VTM, viral transport media.

^a Current FDA-cleared NAATs for CT and GC include: Roche Amplicor CT and GC (Roche Molecular Diagnostics, Indianapolis, IN); APTIMA Combo2 (Gen-Probe, San Diego, CA), BD ProbeTec (Becton Dickinson, Sparks, Md), and Xpert CTNG (Cepheid, Sunnyvale, CA).

^b Pharynx and rectal specimens in MSM (requires laboratory validation for those specimen types).

^c NAAT- nucleic acid amplification test; APTIMA *Trichomonas vaginalis* (ATV) test (Gen-Probe, Inc. San Diego, CA) is a recently FDA-cleared test for both screening as well as diagnosis of TV in women. Multiple specimen types can be used. Same specimen and collection device as currently used for Aptima CT/GC NAAT. Testing for males and alternate sites has been validated by some laboratories. Provider needs to check with laboratory for availability. Some laboratories have validated an in-house PCR method. Check laboratory for availability and specimen types allowed.

^d FDA-cleared hybridization tests for CT/GC include: Digene Hybrid Capture II test CT/GC test (Digene, Silver Spring, Md) and PACE 2C (CT/GC) (Gen-Probe, Inc, San Diego, CA). Neither test cleared for urine specimens; Digene test not cleared for males. Sensitivity not equal to NAAT.

^e Not as sensitive as NAATs.

^f Not widely available; reference test for some specimens; sensitivity approximately 70% compared to NAAT.

^g Epithelial cells are required for adequate exam.

^h Gram stain in males only; 10–15 WBC/HPF and intracellular gram-negative diplococci (gnnc)- 95% specific for GC; no intracellular gnnc seen- only 10%–29% specific for GC.

ⁱ Culture allows for antimicrobial susceptibility testing; culture sensitivity may be better when direct inoculation of specimen to selective media with CO₂ tablet at patient bedside; vancomycin in media may inhibit some GC strains.

^j Wet mount for trichomonads requires live organisms to visualize movement; sensitivity 60%.

^k OSOM *Trichomonas* Rapid Test (Genzyme, Diagnostics, Cambridge, MA); 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.

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

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

ⁿ Check with laboratory, some can be maintained and shipped at RT.

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 (TAT) 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 skin and soft tissue infections:

- 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.
-

Table XI-4. Laboratory Diagnosis for Pathogens Associated with Pelvic Inflammatory Disease and Endometritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Mixed anaerobic organisms Vaginal flora Enterobacteriaceae, enterococci Group A ^a and B streptococci <i>Mycoplasma</i>	Blood cultures and antimicrobial susceptibilities to assess unusual causes of PID or endometritis Gram stain Aerobic and anaerobic culture ^{a,b} Histology for evidence of endometritis	Blood, 2 separate 20 mL venipuncture collections Endometrium, tubo-ovarian abscess and/or fallopian tube contents Endometrial biopsy ^c	Inject into blood culture bottles at bedside, RT, 1 h Place in or inject into sterile anaerobic container ³ , RT, 30 min Sterile container, RT, 30 min Formalin container, RT, 30 min–4 h
<i>Neisseria gonorrhoeae</i> (GC) <i>Chlamydia trachomatis</i> (CT) <i>Trichomonas vaginalis</i>	NAAT	Urine, endocervical swab	Laboratory-provided transport device, RT, 2 d
Human immunodeficiency virus (HIV)	HIV EIA-antibody	Serum	Clot tube, RT, 2 h

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

^a Gram stain may aid in identification of significant pathogen.

^b Limited identification and antimicrobial susceptibility testing (AST) when cultures show multiple mixed aerobic and anaerobic organisms.

^c Invasive specimens obtained by laparoscopic exam.

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 XII-1). 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 which grafting is necessary. Prior to any sampling or biopsy, the wound should be thoroughly cleansed and devoid of topical antimicrobials that can affect culture results. Blood cultures should be collected for detection of systemic disease secondary to the wound.

The application of nucleic acid amplification tests (NAAT) for detection of listed viruses is commonly restricted to blood and/or body fluids. It is advisable that the clinician determine if the local supporting laboratory has validated such assays and if the laboratory has assessed the performance with tissue

specimens. This precaution would also apply to the molecular detection of MRSA (except for one FDA-cleared test for *S. aureus* and MRSA from SSTIs) and VRE [192, 193].

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 XII-2). Major limitations of swabs versus

Table XII-1. Laboratory Diagnosis of Burn Wound Infections

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

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

^a Electrical burns; potential for transmission from leaches.

^b Xpert MRSA/SA SSTI (Cepheid, Sunnyvale, CA).

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 [194–196].

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 dependant upon the type of animal that inflicted the bite (Table XII-3). Since dogs and cats account for the majority of animal-inflicted bite wounds, the two most prominent groups of microorganisms initially considered in the evaluation of patients are *Pasteurella* spp, namely *P. canis* (dogs) and *P. multocida* subspecies *multocida* and subspecies *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 XII-3 is of little benefit since these organisms are not included in most of the commercial identification systems (conventional and automated) data bases [3, 197–206].

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 XII-4). It is strongly recommended that specimens not be submitted for culture within the first 48 hours post-trauma since 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 post-debridement of the trauma site [207–210]. It is recommended that initial cultures focus on common pathogens with additional testing being reserved for uncommon or rare infections associated with special circumstances (ex: detection of *Vibrio* spp following salt-water exposure) or patients with chronic manifestations

Table XII-2. Laboratory Diagnosis of Human Bite Wound Infections

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

Abbreviation: RT, room temperature.

^a No utility in collecting a specimen at the time of the bite; collect samples only if infection occurs.

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 (IVDU) 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 XII-4, with the addition of *Clostridium sordellii*, *C. botulinum* (causing wound botulism), and the agents of human bite wounds (Table XII-2) 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 Centers for Disease Control and Prevention Guidelines for Prevention of Surgical Site Infections, 1999, for specific definitions of SSIs (<http://www.cdc.gov/nhsn/pdfs/pscmanual/9pscscscurrent.pdf>). Of the microbial agents listed below (Table XII-5), *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), coagulase-negative staphylococci, and enterococci are isolated from nearly 50% of these infections [211]. Although nterococcal 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. The recommended IDSA therapeutic regimens for surgical site infections are not reliably active against these organisms [191]. 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, drain insertions, embolizations, thrombolysis, biopsy, radiofrequency ablation, cryoablation, line insertion, inferior vena cava filters, vertebroplasty, nephrostomy placement,

Table XII-3. Laboratory Diagnosis of Animal Bite Wound Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Times
Bacterial^a			
<i>Actinobacillus</i> spp	Aerobic/anaerobic culture	Tissue/biopsy/aspirate	Anaerobic transport container ^b
<i>Capnocytophaga</i> spp			
<i>Erysipelothrix rhusiopathiae</i>	Gram stain		Be certain to provide sufficient volume of sample for complete culture and Gram stain evaluation; RT, <2 h
<i>Pasteurella</i> spp			
<i>Streptobacillus</i> spp			
	Blood culture	Blood; 2–4 cultures per 24 h	Blood culture bottles, RT, <2 h
<i>Mycobacterium fortuitum</i>	Aerobic culture	Tissue/biopsy/aspirate	Sterile container, RT, <2 h
<i>M. kansasii</i>	Acid-fast culture		
	Acid-fast stain		
	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*, CDC Group EF-4, CDC NO-1, *Peptococcus* spp, Rabies or other viruses (refer to Viral Section XIV).

^b Anaerobic transport media preserve all other organisms for culture.

radiologically inserted gastrostomy, dialysis access and related intervention, transjugular intrahepatic porto-systemic 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 intraabdominal 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 one 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 more than 3 days may be difficult due to the presence of colonizing bacteria and yeast.

Drains are characterized as Gravity, Low-Pressure Bulb Evacuators, Spring Reservoir, Low Pressure or High Pressure. Fluids from drains are optimal specimens for collection and submission to the microbiology laboratory. All fluids should be collected aseptically and transported to the laboratory in an appropriate device such as blood culture bottle (aerobic), sterile, leak proof container (ie, urine cup), or a citrate-containing blood collection tube to prevent clotting in the event that blood is present. Expected pathogens from gravity drains originate from the skin or GI tract; for the remaining drain types, skin flora represent the predominate pathogens.

G. Cutaneous Fungal Infections

The presence of fungi (moulds 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 XII-6), eg dermatophytes typically produce tinea (ringworm)-type infections; dematiaceous (darkly pigmented moulds 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 moulds (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 Mason 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 mould 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 [212, 213].

XIII. TICKBORNE INFECTIONS

The clinical microbiology tests of value in establishing an etiology of various tickborne diseases are presented below (Table XIII-1). *Borrelia* species are responsible for relapsing fever and Lyme borreliosis; both diseases are transmitted by ticks to humans. Lyme borreliosis is a multisystem disease that can affect the skin, nervous system, the joints, and heart; this infection is the most frequently reported tickborne disease in the northern hemisphere [151]. For the most part, early Lyme disease is diagnosed on clinical grounds including the presence of erythema migrans while early/disseminated and late/persistent Lyme disease are diagnosed by two-tiered serological testing (EIA followed by Western blot). Western blot should not be performed except as a reflex test after an initial EIA has

Table XII-4. Laboratory Diagnosis of Trauma-Associated Cutaneous Infections

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

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

^a There is an FDA-cleared NAAT for direct detection of *S. aureus* and MRSA 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.

returned positive or equivocal. The IgM Western blot is not clinically interpretable after a patient has had 6–8 weeks of symptoms. Western blot IgG and IgM are based on testing 10 IgG bands and 3 IgM bands. Criteria for positivity are at least 5 IgG bands or at least 2 IgM bands (plus a positive or equivocal EIA) [214]. A ‘post-treatment’ Lyme disease syndrome may occur after appropriate antibiotic therapy for laboratory documented *B. burgdorferi* infection. Persistent symptoms lasting more than six months such as fatigue, musculoskeletal pains, and neurocognitive dysfunction do not permanently respond to long-term antibiotic therapy based on randomized-controlled trial data.”

Rickettsial diseases that are transmitted by ticks include Rocky Mountain spotted fever, human granulocytotropic anaplasmosis, human monocytotropic ehrlichiosis, and others

including those caused by *Ehrlichia ewingii* [215, 216]. Although clinically similar, these diseases are epidemiologically and etiologically distinct illnesses. 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. In addition to Lyme borreliosis and rickettsial diseases, babesiosis and Colorado tick fever virus are also transmitted by ticks.

Since the organisms transmitted by ticks are infrequently encountered in clinical specimens, most clinical microbiology laboratories do not provide all of the services listed in the table below. Of significance, tick borne relapsing fever, ehrlichiosis, anaplasmosis, and babesiosis can all be rapidly diagnosed by examining peripheral blood smears. However, a negative smear

Table XII-5. Laboratory Diagnosis of Surgical Site Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Times
Bacterial			
<i>S. aureus</i>	Gram stain	Tissue/biopsy/aspirate	Keep tissue moist; aerobic transport, RT, <2 h
Coagulase-negative staphylococci	Aerobic culture and AST		
β -hemolytic streptococci (Group A, B, C and G)	NAAT ^a		
Nonhemolytic streptococci	Anaerobic culture (if appropriate)	Tissue/biopsy/aspirate	Anaerobic transport device RT, <2 h
Enterococci			
<i>Acinetobacter</i> spp	Blood culture	Aerobic and anaerobic bottles	RT, <2 h
<i>Pseudomonas aeruginosa</i>			
Enterobacteriaceae			
Indigenous/exogenous aerobic/anaerobic flora	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2 h–24 h RT, indefinite
<i>Mycoplasma hominis</i> and <i>Legionella pneumophila</i> (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
<i>Mycobacterium</i> spp-rapid growers	Acid-fast stain and culture	Tissue/biopsy/aspirate	Aerobic transport device Sterile container RT, <2 h
Fungi			
<i>Candida</i> 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 h–24 h

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

^a There is an FDA-cleared NAAT for direct detection of *S. aureus* and MRSA from swabs of wounds and pus.

^b *M. hominis* has caused infections post-joint surgery and post-abdominal surgery, particularly after caesarian sections. 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.

result does not necessarily rule out a tick borne disease due to the often low and variable sensitivity of peripheral blood smear examination for these organisms. Therefore, 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. 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 turn-around time.

Key points for the laboratory diagnosis of tickborne infections:

- Tick-borne diseases are difficult to diagnose because symptoms are nonspecific, including fever, chills, aches, pains, and rashes.
- Patient travel history, recent locations, and potential for tick bite are important.
- Consultation with the microbiology laboratory is normally required to determine the specimens accepted, the location of the testing laboratory, and the turnaround time for results.

Table XII-6. Laboratory Diagnosis of Fungal Infections of Skin and Subcutaneous Tissue

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

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

XIV. VIRAL SYNDROMES

This section will cover viral infections most commonly encountered in the U.S., realizing that there are a myriad of viruses that can cause illness in humans. Clinical microbiology laboratory tests of value in establishing a diagnosis of viral infections are outlined below. Tests for human immunodeficiency virus

(HIV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), human herpes virus-6 (HHV-6), parvovirus (erythrovirus) B19, measles, mumps, rubella, BK virus, JC virus, dengue, hepatitis A virus, hepatitis B virus (and hepatitis D virus), hepatitis C virus (HCV), enteroviruses, respiratory syncytial virus (RSV), influenza virus, West Nile virus (and other encephalitides),

Table XIII-1. Laboratory Diagnosis of Tickborne Infections

Etiologic Agents ^a	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Times
Bacteria			
Relapsing fever borreliae (4–6)	Primary test ^b : Darkfield microscopy or Wright's, 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
<i>Borrelia hermsii</i> (western USA)			
<i>Borrelia parkeri</i> (western USA)			
<i>Borrelia turicae</i> (southwestern USA)	Others Tests		
<i>Borrelia mazzottii</i> (southern USA)	NAAT	Serum, blood, body fluids	Clot tube for serum; sterile tube or citrate tube for body fluids, RT, within 2–4 h
	Culture ^c		
	Serologic testing ^d	Blood, body fluids Serum	
<i>Borrelia burgdorferi</i> sensu lato complex (Lyme borreliosis) ^e	Early Lyme disease – presence of erythema migrans:	Serum	Clot tube, RT, ≤2 h
<i>Borrelia burgdorferi</i> (USA)	Serologic testing insensitive in the first 2 wk of infection ^f		
<i>Borrelia garinii</i> (Europe, Asia)	Early/disseminated (weeks through months after tick bite) or late/persistent (months through years after tick bite in untreated patients, almost exclusively seen with <i>B. afzelii</i> ^g): Primary test: Two-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 blot (WB). ^h NOTE: A Western blot should NOT be performed unless an initial EIA is reported as positive or equivocal. Neuroborreliosis ^p Paired serum/CSF antibody levels, ie, CSF/serum antibody index. NAAT ⁱ	Serum and CSF	Clot tube for serum, sterile tube for CSF, RT, ≤1 h
<i>Borrelia afzelii</i> (Europe, Asia)			
		Blood, biopsy specimens of infected skin, synovial fluid or tissue, CSF, etc.	Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen at –70°C.

adenovirus, rabies virus and lymphocytic choriomeningitis virus are specifically highlighted. Not all clinical microbiology laboratories provide all of the services outlined in the tables, especially in the case of serologic and molecular tests. When the recommended testing is not available in a local laboratory, it can usually be referred to a reference laboratory with an ensuing possible increase in the time necessary to obtain results.

Specific IgM assays for a variety of viral agents may be associated with false positive results, especially with high titers of IgG antibodies. Therefore, if the pretest probability of acute infection is low to moderate, it is good practice to measure IgG (or total –IgG plus IgM) antibodies at disease presentation (“acute

phase”) and two to three weeks later (“convalescent phase”) to assess for a four-fold or greater rise in antibody titer.

Many molecular diagnostic tests for viral pathogens are laboratory developed tests, offered by Clinical Laboratory Improvement Amendments (CLIA)-certified reference laboratories. Although such tests require establishment of performance characteristics prior to clinical use and appropriate quality systems, performance may vary between laboratories. Throughout this section, the term NAAT generally refers to polymerase chain reaction (PCR) or reverse transcriptase PCR. Other specific techniques may be substituted with appropriate validation.

Table XIII-1 continued.

Etiologic Agents ^a	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Times
<i>Anaplasma phagocytophilum</i> (human granulocytotropic anaplasmosis) ^j	Primary Test: 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
	Acute and convalescent IFA titers for <i>Anaplasma</i> antibodies; specificity ranges from 83% to 100% with cross-reactivity among <i>E. chaffeensis</i> and <i>A. phagocytophilum</i> antibodies, as well as a number of clinical conditions such as Rocky Mountain Spotted Fever, typhus, Q fever, Lyme disease, etc ^k	Serum	Clot tube, RT, ≤2 h
	NAAT	Blood	EDTA anticoagulant tube Transport on ice; ≤1 h
	Immunohistochemical staining of <i>Anaplasma</i> antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)	Formalin container, RT, ≤2 h
<i>Ehrlichia chaffeensis</i> (human monocytotropic ehrlichiosis)	Primary Test: Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection.	Blood	EDTA anticoagulant tube, RT, ≤1 h
<i>Ehrlichia ewingii</i> ^{i,k}	Serology: acute and convalescent IFA titers for <i>Ehrlichia</i> antibodies ^l	Serum	Clot tube, RT, ≤2 h
	NAAT (only definitive diagnostic test for <i>E. ewingii</i>)	Whole blood	Heparin or EDTA anticoagulant tube Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen.
	Immunohistochemical staining of <i>Ehrlichia</i> antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)	Formalin container, RT, ≤2 h
<i>Rickettsia rickettsii</i> (Rocky Mountain spotted fever) ^{m,n}	Serology: acute and convalescent IFA for <i>R. rickettsii</i> IgM and IgG antibodies ^l	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			
<i>Babesia microti</i>	Primary Test: Giemsa, Wright's, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood	For whole blood, prepare smears immediately
		Second choice EDTA vacutainer tube	RT, ≤30 min
	NAAT	Blood	EDTA anticoagulant tube, RT, ≤1 h
	Serology: acute and convalescent IFA titers for <i>Babesia</i> antibodies (IgM and IgG)	Serum	Clot tube, RT, ≤2 h

Table XIII-1 continued.

Etiologic Agents ^a	Diagnostic Procedures	Optimum Specimens	Transport Issues; 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 ^o	Serum	Clot tube, RT, ≤2 h

Abbreviations: IFA, indirect fluorescent antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

^a Other tick-borne diseases should be considered if patients have traveled to international destinations. Since 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 world-wide and might have epidemiologic, seasonal and clinical features that differ from those observed in the U.S. [215]. 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 [217].

^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* species that cause relapsing fever. A centrifugation-based enrichment method followed by Giemsa staining is a rapid and viable approach [218].

^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, three 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 [219]. A "chronic" or "post" Lyme disease syndrome after initial short-course antibiotic treatment has not been supported in a rigorous scientific study. Treatment of "chronic Lyme disease" is a controversial issue that has been addressed by IDSA in a guideline available on its website (<http://cid.oxfordjournals.org/content/43/9/1089.full#sec-36>).

^f Erythema migrans (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 [220]. If skin is biopsied, more than 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.

^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* species; in Europe; coinfection with tick-borne encephalitis virus should also be considered [221].

^h Perform an IgM and an IgG 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 greater than 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 longer than 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 Other Lyme-associated diseases can be diagnosed by NAAT (TAT 24–48 hours) or culture (TAT 3 days to 6–12 weeks). Acceptable specimens for multiple erythematous or borrelial lymphocytoma, Lyme carditis, Lyme arthritis, and acrodermatitis are skin biopsy, endomyocardial biopsy, synovial fluid or biopsy, and skin biopsy, respectively [221, 223]. Although *Borrelia* can be detected by NAAT in blood, biopsy specimens of infected skin, synovial tissue or fluid, or CSF, its usefulness for the diagnosis of Lyme disease is limited at this time. For example, *Borrelia* DNA is detected in the blood of fewer than half of patients in the early acute stage of disease when the erythema migrans 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 erythema migrans can be reasonably diagnosed and treated on the basis of history and clinical signs alone.

^j 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 intracytoplasmic inclusions (morulae) in either monocytes or neutrophils or bands.

^k A newly discovered *Ehrlichia* species was reported to cause ehrlichiosis in Minnesota and Wisconsin; this *Ehrlichia* is closely related to *Ehrlichia muris* [224].

^l 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% to 100% sensitive after 14 days of onset of symptoms and sensitivity is increased if paired samples are tested.

^m 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 Rocky Mountain spotted fever (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.

ⁿ Antibiotic therapy may diminish the development of convalescent antibodies in RMSF.

^o IgM antibodies develop 2 weeks after symptom onset.

^p Laboratory assays for the diagnosis of neuroborreliosis are of limited clinical value [151, 222].

Key points for the laboratory diagnosis of viral syndromes:

- Viral syndromes should be considered based on the patient's age, immune status, history, and many other variables.
 - Samples can be obtained and tested for the most likely agents, with additional samples held frozen in the laboratory for additional testing if necessary; 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 virology capabilities and tests will be sent out, resulting in longer turnaround times for results.
 - Cross-reactivity among some agents will result in non-specific serologic results.
 - Tests for immunity, previous virus infection (eg, tissue donors), and new infection may have different formats, even when the same virus is being considered.
-

A. Human Immunodeficiency Virus (HIV)

HIV-1 is an RNA virus with a genome consisting of three major genes encoding capsid proteins (*gag* – p55, p24, p17); reverse transcriptase, protease, and integrase (*pol* – p66, p51, p31); and envelope glycoproteins (*env* – gp160, gp120, gp41). HIV viruses are classified based on relatedness of genomic sequence into types 1 and 2, groups, and clades. HIV-1 and HIV-2 proteins differ in molecular weight. HIV-1 is categorized into groups M, O, non-M, non-O (N) and P, with M being most common [225, 226]. HIV-1 is more common than HIV-2 in the U.S.; 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.

Antibodies are detectable in acute HIV infection, usually within the first four weeks following exposure, preceded in positivity by p24 antigen, which is in turn preceded (by three to five days) by HIV RNA positivity. Performing an HIV RNA test after a negative initial antibody and/or antigen test in persons suspected of acute infection may therefore be helpful. Because of the time course of test positivity and the possibility of seronegativity, the 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 XIV-1) [227]. Outside of the setting of acute HIV infection however, HIV viral load assays should be used with caution for diagnosis of HIV infection because of the possibility

of false positive results. False positive results are generally of low copy number (<5000 copies/mL); therefore, low copy number results should prompt retesting of a second specimen. Notably, because there is a 10- to 14-day period after infection when no markers are detectable, testing another specimen two to four 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, it may be helpful to document subsequent HIV-1 seroconversion by conventional serologic testing.

In the neonate, serologic testing is unreliable due to persistence of maternal antibodies; quantitative HIV-1 RNA (viral load) testing is as sensitive as qualitative HIV-1 RNA and/or proviral DNA testing for the diagnosis of HIV-1 infection [228]. Serologic diagnosis has evolved since the 1980s. First and second generation assays were indirect EIAs that used viral lysate and recombinant/synthetic peptide antigens, respectively. Third generation assays allowed detection of HIV IgM (in addition to IgG), enabling earlier diagnosis of infection. The most recent—fourth generation—assays incorporate HIV p24 antigen detection, allowing even earlier diagnosis of infection. Third and fourth generation assays are generally positive seven to 14 and four to seven days, respectively, after detectable virus by NAAT.

HIV p24 antigen may be detected in serum or plasma between 14 and 22 days after infection (before antibody becomes detectable); it typically decreases below detection limits thereafter, limiting utility of p24 antigen testing alone. Combined HIV antibody plus p24 assays (ie, fourth generation assays) are in widespread use as initial screening assays and the Association of Public Health Laboratories and the Centers for Disease Control and Prevention now recommend them as initial screening tests for diagnosis of HIV infection [225, 226]. The testing algorithm associated with their use does not require Western Blot. Instead, individuals with reactive results are further tested with an antibody immunoassay that distinguishes HIV-1 from HIV-2 antibodies. If the differentiation assay 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 determination) is recommended to direct management. An alternate approach is an initial HIV antigen/antibody combination assay that discriminates detection of antigen from antibody; p24 reactivity is subsequently confirmed by NAAT and antibody reactivity by an HIV-1/HIV-2 differentiation assay. The traditional laboratory diagnosis of nonacute HIV-1 infection (Table XIV-1) begins with screening for HIV-1/-2 antibodies. When testing by the screening assay shows reactive results, confirmatory testing by Western blot is

performed. (As an alternative, NAAT testing or a second antibody immunoassay—using different antigenic constituents or based on different principles—may be considered.) An initial positive HIV antibody immunoassay using an oral fluid specimen is followed by an immunoassay performed on blood, serum or plasma, a positive result of which is followed by NAAT or supplemental antibody testing to corroborate infection. A negative blood, serum or plasma result requires follow-up testing as previously described [225, 226]. Using the traditional algorithm, if the Western blot result is positive, the patient is considered to be infected with HIV-1. If negative, testing for HIV-2 antibodies is recommended to rule out the possibility of HIV-2 infection causing the reactive combined HIV-1/-2 antibody result. If the Western blot test is unreadable (ie, due to high background reactivity of the strip), testing with an HIV-1 antibody-specific indirect immunofluorescence assay or qualitative or quantitative testing for HIV-1 RNA or for proviral DNA should be considered. According to Association of Public Health Laboratories and the Centers for Disease Control and Prevention, an HIV-1 antibody Western blot result is interpreted as positive when at least 2 of the 3 following bands are present: p24, gp41, and gp120/gp160. If only one of these bands is present, the result is indeterminate, and additional supplemental testing with an HIV-1 antibody-specific indirect immunofluorescence assay, an EIA for HIV-2 antibodies alone, and a qualitative HIV-1 RNA and/or proviral DNA assay should be considered. Causes of indeterminate Western blots include evolving antibody profiles, specimen contamination, antibody decline with immune system failure (late stage infection), nonspecific reactivity due to viral or cellular protein components, other infections (eg, syphilis, other retroviruses, some parasites), immune-modulating conditions (eg, pregnancy), and infection with groups N, O, or P HIV-1, or HIV-2. High-risk patients with reactive serologic screening test results and indeterminate Western blots but negative supplemental tests should be considered for retesting two to four weeks later. If this does not resolve the issue, additional supplemental testing (eg, NAAT) may be considered. Western blot assay is less sensitive than third or fourth generation EIAs during seroconversion with up to three weeks following a positive fourth generation assay before a positive Western blot assay. Since as many as a third of healthy HIV-uninfected blood donors have indeterminate HIV-1 Western blot assays, they should not be ordered as the first test for HIV. Line immunoassays incorporating HIV-1 and HIV-2-specific recombinant proteins and/or synthetic peptides (compared to purified proteins separated by electrophoresis used in Western blotting) are alternatives to Western blot assays.

Resistance testing is recommended for patients with acute or chronic HIV infection prior to initiating therapy (including treatment-naïve pregnant HIV-1-infected women), virologic

Table XIV-1. Laboratory Diagnosis of Human Immunodeficiency Virus (HIV)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Initial screening tests for HIV-1/2 antibodies plus HIV-1 p24 antigen or HIV-1/2 antibodies alone	Serum Plasma ^a	Clot tube RT, <2 h
Western blot for HIV-1 or HIV-2 antibodies		
Line immunoassays for HIV-1 or HIV-2 antibodies		
Rapid point-of-care tests for HIV antibodies	Oral fluid (saliva), whole blood (fingerstick, venipuncture), urine Serum Plasma ^a	Clot tube RT, <2 h
HIV-1 RNA detection, qualitative	Plasma ^a	EDTA, RT, <2 h
HIV-1 RNA quantification (viral load)	Plasma ^{a,b}	EDTA, RT, <2 h
HIV-1 proviral DNA, qualitative ^c	Whole blood, EDTA or citrate	EDTA or citrate, RT, <2 h
HIV-1 RNA and proviral DNA, qualitative	Whole blood ^a	EDTA, RT, <2 h
HIV-1 resistance testing, genotypic or phenotypic	Plasma ^a	EDTA, RT, <2 h
HIV-2 RNA and proviral DNA, qualitative ^d	Whole blood ^a	EDTA, RT, <2 h

Abbreviation: RT, room temperature.

^a Do not transport in plasma preparation tube. The specimen should be collected in a lavender top (EDTA) tube.

^b Methods used for quantification of HIV RNA include target amplification (eg, reverse transcriptase polymerase chain reaction, transcription-mediated amplification, and nucleic acid sequence-based amplification) and signal amplification (eg, branched DNA) assays.

^c Plasma should be promptly removed from cells after collection to prevent leakage of proviral DNA from cells. Since PCR does not differentiate plasma virion RNA from proviral DNA, leakage of proviral DNA from cells may result in falsely elevated plasma HIV RNA viral load. Signal amplification assays (eg, branched DNA assays) are not similarly affected.

^d Consider HIV-2 in those with a clinical status suggestive of AIDS but a nonreactive HIV-1 immunoassay result, discordant results between an HIV-1 and HIV-1/HIV-2 immunoassays, a reactive HIV-1/HIV-2 immunoassay result with a negative or indeterminate HIV-1 Western blot assay, or confirmed HIV infection by undetectable off-treatment HIV-1 viral load.

failure during combination drug therapy, and suboptimal suppression of viral load after initiating therapy.

B. Epstein-Barr Virus

Epstein-Barr virus is a cause of mononucleosis 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 results may be found in patients with leukemia, pancreatic carcinoma, viral hepatitis, CMV infection, etc. False-negative results are obtained in approximately 10% of patients, and are especially common in children younger than 10 years.

When rapid Monospot or heterophile test results are negative, additional laboratory testing (Table XIV-2) may be considered to differentiate EBV infection from a mononucleosis-like illness caused by CMV, adenovirus, HIV, *Toxoplasma gondii*, etc. In this situation, EBV antibody testing for IgG and IgM to viral capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA) are recommended. The presence of VCA IgM (with or without VCA IgG) antibodies in the absence of antibodies to EBNA indicates recent primary infection with EBV. The presence of EBNA antibodies indicates infection more than 6 weeks from the time of the sample and therefore not likely implicating EBV as a cause. Antibodies to EBNA develop one to two or more months after primary infection and are detectable for life. Over 90% of the normal 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 the EBNA antigen.

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. Increases in EBV viral load detected by NAAT in peripheral blood may be present in patients before the development of EBV-associated lymphoproliferative disease; these levels typically decrease with effective therapy. Tissues from patients with EBV-associated lymphoproliferative disease may show monoclonal, oligoclonal, or polyclonal lesions. The diagnosis of EBV-associated lymphoproliferative disease requires demonstration of EBV DNA, RNA or protein in biopsy tissue.

NAAT may be used to detect EBV DNA in CSF of patients with acquired immunodeficiency syndrome-related central nervous system lymphoma, however EBV DNA may also be present in cerebrospinal fluid with other abnormalities (eg, central nervous system toxoplasmosis, pyogenic brain abscesses) and therefore positivity is nonspecific. Detection of antibody in CSF may indicate central nervous system infection, blood contamination, or transfer of antibodies across the blood-brain barrier. Calculation of the CSF to serum antibody index may be helpful.

C. Cytomegalovirus

In immunocompetent individuals suspected of having acute CMV infection, testing for CMV-specific antibodies is recommended as the first line laboratory diagnostic test (Table XIV-3). In the immunocompetent host, the presence of IgM class antibodies indicates recent infection; however, false positive CMV IgM results may occur in patients infected with EBV or with activated immune systems due to other causes. The presence of IgG antibodies alone indicates past exposure to CMV.

Table XIV-2. Laboratory Diagnosis of Epstein-Barr Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Heterophile antibody test or Monospot	Serum	Clot tube, RT, <2 h
IgG and IgM to viral capsid antigen, and antibodies to Epstein-Barr nuclear antigen	Serum	Clot tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
EBV DNA quantification (viral load)	Whole blood, peripheral blood lymphocytes, plasma	EDTA, RT, <2 h
	Cerebrospinal fluid	
EBV DNA detection, qualitative	Cerebrospinal fluid	Sterile tube, RT, <2 h

Abbreviations: EBV, Epstein-Barr virus; IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

Table XIV-3. Laboratory Diagnosis of Cytomegalovirus (CMV)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
Antigenemia (direct counting of stained cells; method no longer considered optimal)	Whole blood	Blood tube with heparin, EDTA, or citrate anticoagulant RT, <2 h
CMV DNA quantification (viral load)	Plasma, whole blood	EDTA anticoagulant tube, RT, <2 h
	Cerebrospinal fluid	Sterile container, RT, <2 h
CMV DNA detection, qualitative	Cerebrospinal fluid, urine, tissues, respiratory specimens, body fluids	Sterile container, RT, <2 h
Culture	Urine	Sterile container, RT, <2 h

Abbreviation: RT, room temperature.

In recipients of organ or peripheral blood stem cell transplants, CMV viral load by NAAT or antigenemia (performed by fewer laboratories as NAATs gain favor) is used as a marker for preemptive therapy, to diagnose CMV-associated signs and symptoms, and to monitor response to antiviral therapy. Standard Reference Material (SRM) is available from the National Institute of Standards and Technology (NIST) 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/microliter).

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 waiting time can be shortened 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. Because 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 sequencing of *UL97* (phosphotransferase gene) with or without *UL54* (DNA polymerase gene) is utilized in such situations. Sequencing-based assays are performed on DNA amplified directly from clinical specimens, provided they contain a sufficient quantity of CMV DNA. Alternatively, the virus can first be isolated in cell culture. Ganciclovir resistance most commonly emerges due to point mutations or deletions in *UL97* (with foscarnet and cidofovir unaffected) with mutations at three 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; but 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-central nervous system infection, but false positive results may occur (eg, in patients with bacterial meningitis in whom CMV DNA in blood crosses the inflamed blood-brain barrier and contaminates cerebrospinal fluid). Detection of antibody in cerebrospinal fluid may indicate central nervous system infection, blood contamination, or transfer of antibodies across the blood-brain barrier. Calculation of the CSF to serum CMV antibody index may be helpful.

D. Varicella-Zoster Virus

The presence of VZV IgG and IgM typically indicates recent infection with VZV; however, these results may also be observed in patients with recent immunization to VZV. A positive VZV IgG with a negative VZV IgM result indicates 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 specimen may have been drawn before the appearance of detectable antibodies. Negative results in suspected early VZV infection should be followed by testing a new serum specimen in two to three weeks.

The most sensitive and specific test for diagnosis of VZV-associated skin lesions is NAAT (Table XIV-4). A culture transport swab is vigorously rubbed on the base of the suspect skin lesion; the vesicle may be unroofed to expose the base. A less sensitive method for diagnosis is detection of viral antigens by direct fluorescent antibody stain of lesion scrapings. VZV culture is not recommended since this virus is difficult to grow in routine cell lines and may take two weeks to isolate (unless using shell vial assay). Suspected VZV-associated skin lesions must be clinically differentiated from smallpox as described in the algorithm developed by the Centers for Disease Control and Prevention (<http://www.bt.cdc.gov/agent/smallpox/diagnosis/riskalgorithm/index.asp>); information about laboratory testing for smallpox is available at <http://www.bt.cdc.gov/agent/smallpox/lab-testing>.

VZV NAATs can be performed on CSF as an aid to the diagnosis of VZV central nervous system infection. CSF IgM or intrathecal antibody synthesis distinguishes meningoencephalitis from a post-infectious immune-mediated process.

E. Herpes Simplex Virus

The presence of IgG antibodies specific to the glycoprotein G antigen from HSV type 1 or 2 indicates previous exposure to

Table XIV-4. Laboratory Diagnosis of Varicella-Zoster Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues (RT); Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
NAAT	Scraping of base of dermal lesion collected using swab	Viral transport medium ^a RT, <2 h
	Cerebrospinal fluid, sterile tube	RT, <2 h
Direct fluorescent antibody test	Vesicle fluid on slide	Place in sterile container, RT, <2 h

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

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

Table XIV-5. Laboratory Diagnosis of Herpes Simplex Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
NAAT	Scraping of base of dermal or mucosal lesion collected using a swab	Place into viral transport medium ^a RT, <2 h
	Cerebrospinal fluid	Sterile tube, 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 ^a RT or on wet ice, <2 h

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

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

the corresponding serotype of the virus. Positive IgG results do not differentiate past from current, active infection unless seroconversion is determined by testing, in parallel, acute and convalescent phase specimens. A fourfold increase in IgG results may also suggest recent exposure; however, most commercial assays no longer yield a titered result that can be used quantitatively. The presence of IgM antibodies to HSV suggests active, primary infection with this virus.

NAAT is the most sensitive, specific and rapid test for diagnosis of HSV-associated skin or mucosal lesions and should detect and distinguish HSV types 1 and 2 (Table XIV-5). A viral culture transport swab is 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.

HSV NAATs performed on CSF are used to diagnose HSV central nervous system disease [229]. The assay should detect and distinguish HSV types 1 and 2; type 1 is most commonly associated with encephalitis and type 2 with meningitis. Viral culture of cerebrospinal fluid is insensitive for diagnosis of HSV central nervous system disease.

F. Human Herpes Virus-6

Human herpes virus-6 causes roseola infantum in children and can cause primary or reactivation infection in immunocompromised patients. IgG seroconversion, the demonstration of anti-HHV-6 IgM, or a four-fold rise in IgG antibodies from paired sera may indicate recent infection with HHV-6. Commercial

Table XIV-6. Laboratory Diagnosis of Human Herpes Virus-6

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
NAAT	Serum	Serum: Clot tube RT, <2 h
	Plasma	Plasma or whole blood: EDTA tube, RT, <2 h
	Whole blood	
	Peripheral blood mononuclear cells	
	Saliva	Sterile container, RT, <2 h
	Cerebrospinal fluid	Sterile container, RT, <2 h

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

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 two years of age. Therefore, a single HHV-6 IgG serologic test result may not be clinically relevant. Five percent of asymptomatic adults may be IgM positive at any given time.

The most commonly used molecular test for the laboratory diagnosis of HHV-6 is NAAT (none FDA-cleared), some formats of which differentiate variants A and B (Table XIV-6). 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 whole blood, may lead to an erroneous diagnosis of active infection. HHV-6 can be cultured from peripheral blood mononuclear cells (and other clinical specimens) [230]. However, viral isolation is labor-intensive, taking up to 21 days; the detection time can be shortened to 1–3 days with the 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. HHV-6 antigens can be demonstrated by immunohistochemical or in situ hybridization tests in formalin-fixed, paraffin-embedded tissues.

G. Parvovirus (Erythrovirus) B19

In immunocompetent individuals with erythema infectiosum or arthralgia/arthritis, testing for parvovirus (erythrovirus) B19-specific antibodies is recommended as the first line laboratory diagnostic method for parvovirus B19 infection (Table XIV-7). The presence of IgM class antibodies suggests recent infection. IgM antibodies can be detected 10 to 14 days post infection and may persist for five months, and occasionally even longer [231]. IgG and IgM reach peak titers within one

Table XIV-7. Laboratory Diagnosis of Parvovirus (Erythrovirus) B19

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bone marrow histopathology	Bone marrow	Sterile container, RT, <2 h Formalin container, RT, 2 h–24 h
Serology	Serum	Clot tube, RT, <2 h
NAAT	Serum	Serum: Clot tube, RT, <2 h
	Plasma or whole blood	Plasma or whole blood: EDTA tube, RT, <2 h

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

month. IgG antibodies may persist for years. The presence of IgG antibodies alone is indicative of past exposure and suggests immunity; this test may be helpful for women in the first trimester of pregnancy. Serologic tests may be negative in the immunocompromised host despite prior exposure to the virus.

Parvovirus B19 DNA-based assays may be used for the diagnosis of parvovirus B19 infection presenting as transient aplastic crisis or chronic anemia in immunosuppressed patients. NAAT is the most sensitive noninvasive technique for the laboratory diagnosis of parvovirus B19-related anemia in solid organ transplant recipients, although current tests are laboratory-validated and not FDA-cleared. A 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. 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

Individuals who are immune to measles should yield a positive result for IgG antibody to the virus. Those who are not immune have negative IgG and IgM results. Recent infection with measles virus is typically indicated by a positive IgM antibody result in the absence of IgG. IgM is often positive on the day of onset of rash; however, in the first 72 hours after rash onset, up to 20% of tests for IgM may be falsely negative. Therefore, if the acute IgM is negative, a second serum specimen, collected 72 hours after rash onset, should be tested for IgM. IgM is detectable for a month or longer after rash onset. IgM may be positive in individuals with recent immunization to measles virus. A serologic diagnosis of acute measles requires demonstration of a four-fold rise in IgG antibody titer (Table XIV-8). Two serum specimens are collected, with the first specimen being obtained as soon as possible after rash onset, and the second specimen being collected 10 to 30 days later; both should then be tested concurrently

Table XIV-8. Laboratory Diagnosis of Measles (Rubeola)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
Culture	Urine	Sterile container, RT, <2 h
	Oropharyngeal or nasopharyngeal swab, ^a nasal aspirate	Viral transport media, RT or on wet ice, <2 h
	Blood	EDTA, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
NAAT	Oropharyngeal swab, oral fluid	Sterile container, RT, <2 h
	Urine	Sterile container, RT, <2 h
	Blood	EDTA tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h

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

^a Place the swab in viral transport medium, cell culture medium or other sterile isotonic solution (eg, saline).

by the same method. Criteria for documenting an increase in titer depend on the specific test used.

Measurement of measles-specific antibodies in CSF is used in the diagnosis of subacute sclerosing panencephalitis (SSPE); levels of rubeola antibody are highly elevated in the cerebrospinal fluid of SSPE patients compared to those without the disease.

Measles virus can be isolated from throat or nasopharyngeal swabs or urine. Specimens should be collected soon after rash onset. NAAT also can be considered as a diagnostic test option [232].

¹Place the swab in viral transport medium, cell culture medium or other sterile isotonic solution (eg, saline).

I. Mumps Virus

Several types of tests are used for mumps diagnosis (Table XIV-9). Laboratory criteria for the diagnosis of mumps include a positive serologic test for mumps IgM antibody, a four-fold rise in serum mumps IgG antibody levels between acute- and convalescent-phase paired sera, isolation of mumps virus from clinical samples, or detection of mumps RNA in a clinical specimen.

Sera for acute phase IgG testing should be collected within 5 days after symptom onset (ie, at the time of diagnosis); convalescent sera should be collected approximately two weeks after symptom onset. IgM antibodies typically become detectable during the first few days of illness and reach a peak about a week after onset. Receipt of one or more doses of the mumps vaccine may result in an absent, delayed or transient IgM response. If the acute IgM is negative, a second specimen should be collected for IgM testing 2–3 weeks after onset of symptoms.

Table XIV-9. Laboratory Diagnosis of Mumps

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
Culture	Parotid (Stensen's) duct/ buccal swab ^a	Sterile container, RT but best on wet ice, <2 h
	Oropharyngeal or nasopharyngeal swab ^b	
	Urine	
	Cerebrospinal fluid	Sterile tube, RT but best on wet ice, <2 h
NAAT	Parotid gland duct/buccal swab ^a	Viral transport medium, RT, <2 h
	Oropharyngeal or nasopharyngeal swab ^b	Viral transport medium, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h

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

^a Massage parotid gland for 30 seconds and then swab parotid (Stensen's) duct using a viral culture transport swab.

^b Place the swab in viral transport medium, cell culture medium or other sterile isotonic solution (eg, saline).

Among previously immunized suspected cases, mumps virus detection is a particularly important method of confirming the case. The preferred sample for viral isolation is a swab from the parotid duct, or from the duct of another affected salivary gland. Mumps virus can also be detected by molecular techniques (no FDA-cleared tests) [233]. Mumps viral RNA may be detected prior to onset of parotitis until five to nine days after onset.

Detection of antibody in CSF may indicate central nervous system infection, blood contamination, or transfer of antibodies across the blood brain barrier. Calculation of the CSF to serum antibody index to mumps virus may be helpful.

J. Rubella Virus

Serology is the most common method of confirming the diagnosis of rubella (Table XIV-10). The presence of antibodies to rubella virus in a single serum specimen is evidence of immunity. Acute rubella infection can be serologically confirmed by a four-fold rise in rubella IgG antibody titer between acute and convalescent serum specimens or by the presence of serum rubella IgM. If testing is performed, serum should be collected

Table XIV-10. Laboratory Diagnosis of Rubella

Diagnostic Procedure	Optimum Specimen	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h

Abbreviation: RT, room temperature.

Table XIV-11. Laboratory Diagnosis of BK Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Urine cytology	Urine ^c	100 mL urine in 250 mL clear plastic collection bottle containing 50 mL of 2% carbowax solution (Saccomanno's fixative) or alternative fixative 50% ethyl alcohol in equal volume to urine, RT, <2 h
BK virus NAAT quantitative (viral load)	Plasma	EDTA tube, RT, <2 h
	Serum	Clot tube, RT, <2 h
	Urine	Sterile container, RT, <2 h

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

as early as possible (within 7 to 10 days) after onset of illness, and again 14 to 21 days (minimum of 7) days later. Caution should be taken in interpreting positive rubella IgM results, as false positive results can occur. Rubella is no longer endemic in the United States; therefore, IgM testing should only be performed in patients with a clinical presentation suggestive of acute rubella. Prenatal screening for rubella immunity should only be performed using an IgG-based assay.

K. BK Virus

BK virus causes allograft nephropathy in renal transplant recipients, a definitive diagnosis of which requires renal allograft biopsy with in situ hybridization for BK virus. BK virus may also cause hemorrhagic cystitis, especially in stem cell transplant recipients.

Detection of certain levels of BK viral load by NAAT in plasma may provide an early indication of allograft nephropathy, although there are no FDA-cleared NAATs (Table XIV-11) [234]. Urine cytology or quantitative NAAT may be used as a screening test, followed by BK viral load testing by NAAT, if positive. Urine NAAT for BK virus may be more sensitive than urine decoy cell (virus-infected cells shed from the tubules or urinary tract epithelium) detection; BK virus DNA may be present earlier in the urine than are decoy cells. However, urinary shedding of BK virus is a common occurrence; 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 false positive results.

L. JC Virus

JC virus is the etiologic agent of progressive multifocal leukoencephalopathy (PML), an often fatal demyelinating disease of the central nervous system that occurs in immunocompromised

Table XIV-12. Laboratory Diagnosis of JC Virus

Diagnostic Procedure	Optimum Specimen	Transport Issues; Optimal Transport Time
NAAT	Cerebrospinal fluid	Sterile tube, RT, <2 h

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

hosts. Histologic examination of brain biopsy tissue reveals characteristic pathologic changes. In situ hybridization for JC virus may be performed on brain tissue. Detection of JC virus DNA by NAAT in CSF specimens of patients with suspected progressive multifocal leukoencephalopathy has largely replaced the need for tissue biopsy for laboratory diagnosis (Table XIV-12).

M. Dengue Virus

Dengue is a mosquito-borne febrile illness. In travelers from certain areas, Chikungunya and yellow fever should be considered in the differential diagnosis, along with malaria. Dengue diagnosis requires laboratory confirmation by culture, NAAT or testing for dengue specific antibodies (Table XIV-13) [235]. Serologic testing represents the most common method for diagnosis of dengue infection. An acute-phase serum specimen should be collected within five days after onset of fever. Patients in the early stage of dengue fever virus infection may not have detectable IgG antibodies; IgG antibodies typically take at least six days after onset of symptoms to develop. IgG antibodies to dengue may persist for decades. If a negative test result is reported for a patient for whom dengue fever is strongly suspected, a second serum specimen should be drawn 7 to 10 days after disease onset and tested for IgM and IgG antibodies. While detection of dengue IgM may indicate recent infection, seroconversion of dengue IgG should also be demonstrated to confirm the diagnosis. Tests for anti-dengue antibodies may detect antibodies to other flaviviruses, including West Nile and St. Louis encephalitis viruses. Molecular testing for dengue virus is available upon special request from the Centers for

Table XIV-13. Laboratory Diagnosis of Dengue

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
Culture	Serum	Clot tube, RT, <2 h
NAAT	Serum	Clot tube, RT, <2 h
	Plasma	EDTA tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h

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

Table XIV-14. Laboratory Diagnosis of Hepatitis A Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Hepatitis A IgM	Serum	Clot tube, RT, <2 h
Hepatitis A total antibodies	Plasma	EDTA tube, RT, <2 h

Abbreviation: RT, room temperature.

Disease Control and Prevention and selected reference laboratories.

N. Hepatitis A Virus

Diagnosis of acute hepatitis A virus infection is confirmed by detecting hepatitis A-specific IgM antibodies (Table XIV-14). The presence of hepatitis A virus-specific total antibodies (ie, IgM and IgG combined) in an asymptomatic patient with normal liver tests indicates either past hepatitis A infection or immunity to this viral infection from vaccination. Currently, there is no commercially available laboratory test for detecting only hepatitis A-specific IgG antibodies.

O. Hepatitis B, D, and C Viruses

Hepatitis B surface antigen may be detected in the presence of acute or chronic hepatitis B virus infection [236]; it indicates that the person is infectious. In acute infection, its appearance predates clinical symptoms by four weeks and it remains detectable for one to six weeks. The tests for hepatitis B and D disease detection are primarily serologic and molecular (Table XIV-15). Check with the laboratory about minimum volumes of blood needed, as some molecular platforms require more blood than others.

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

Hepatitis B core IgM antibodies appear during acute or recent hepatitis B virus infection and remain detectable for about six months. A serologic “window” occurs when hepatitis B surface antigen disappears and hepatitis B surface antibody is undetectable. During this “window” period, infection can be diagnosed by detecting hepatitis B core IgM antibodies, which can remain detectable for up to six months.

Hepatitis B core total antibodies appear at the onset of symptoms of acute hepatitis B infection and persist for life; their presence indicates acute (mainly virus-specific IgM antibodies), recent (both hepatitis B core-specific IgM and IgG antibodies),

Table XIV-15. Laboratory Diagnosis of Hepatitis B (and D) Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Hepatitis B surface antigen (HBsAg)	Serum	Clot tube, RT, <2 h
Hepatitis B surface antibody (anti-HBs)	Plasma	EDTA, RT, <2 h
Hepatitis B core total antibodies (anti-HBc total)		
Hepatitis B core IgM antibody (anti-HBc IgM)		
Hepatitis B e antigen (HBeAg)		
Hepatitis B e antibody (anti-HBe)		
Hepatitis D total antibodies (anti-HDV total)		
Hepatitis D IgM antibody (anti-HDV IgM)		
Hepatitis D IgG antibody (anti-HDV IgG)		
Hepatitis D antigen		
Hepatitis B virus DNA quantification (viral load)		

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; RT, room temperature.

or previous (hepatitis B core-specific IgG antibodies) hepatitis B infection.

A chronic hepatitis B virus carrier state is defined by persistence of hepatitis B surface antigen for at least 20 weeks. In patients with chronic hepatitis B infection, the presence of hepatitis B e antigen in serum or plasma is a marker of high viral replication levels in the liver. Loss of hepatitis B e antigen and emergence of antibody to hepatitis B e antigen is usually associated with improvement of underlying hepatitis and a reduction in the risk of hepatocellular carcinoma and cirrhosis. Alternatively, disappearance of hepatitis B e antigen may denote the emergence of a precore mutant virus; high concentrations of HBsAg and HBV DNA, in the absence of hepatitis B e antigen and presence of antibody to hepatitis B e antigen suggest the presence of a precore mutant virus. Hepatitis B viral DNA is present in serum or plasma in acute and chronic hepatitis B infection [237]. Quantification of hepatitis B viral DNA (by PCR or branched-DNA assay methods) may be included in the initial evaluation and management of chronic hepatitis B infection, especially when deciding treatment initiation and monitoring patient's response to therapy. Other molecular laboratory tests used in the diagnosis and management of hepatitis B infection have been reviewed and include assays for

determining viral genotype, detection of genotypic drug resistance mutations, and core promoter/precore mutations [237].

Detection of hepatitis B surface antibodies in the absence of hepatitis B core total antibodies distinguishes vaccine-mediated immunity from immunity acquired by natural infection (in which hepatitis B surface and hepatitis B core total antibodies are both present). Current commercially available assays for detecting hepatitis B surface antibody yield positive results (qualitative) for antibody levels of ≥ 10 mIU/mL in serum or plasma, indicating post-vaccination immunity (protective antibody level). Quantitative hepatitis B surface antibody results are used to monitor adequacy of hepatitis B immune globulin therapy in liver transplant recipients receiving such therapy during the post-transplant period.

In acute hepatitis D superinfection of a patient with known chronic hepatitis B, hepatitis D antigen, hepatitis D-specific IgM and total antibodies are present (Table XIV-15). In acute hepatitis B and D co-infection, the same serologic markers (ie, hepatitis D antigen, hepatitis D-specific IgM and total antibodies) are present, along with hepatitis B core IgM antibodies.

The diagnosis of HCV usually begins with a screening test for HCV-specific IgG antibodies using EIA or chemiluminescent immunoassay (CIA). Antibodies may not be detectable, however, until six to ten weeks after the onset of clinical illness. Individuals with negative screening test results do not need further testing for HCV (Table XIV-16). Those with positive screening test results should undergo confirmatory or supplemental testing for HCV RNA by molecular test methods. Signal-to-cut-off ratios (calculated by dividing the optical density value of the sample tested by the optical density value of the assay cut-off for that run) are an alternative to supplemental testing (<http://www.cdc.gov/hepatitis/HCV/LabTesting.htm>). Hepatitis C virus RNA can be detected by NAATs soon after infection as well as in chronic infection. NAAT for HCV can be performed qualitatively (by reverse-transcription PCR or transcription-mediated amplification) or quantitatively (by reverse-transcription PCR or branched DNA). Prior to and during treatment, quantification of HCV RNA (by PCR or branched-DNA assay methods) is necessary to monitor rapid and early virologic response to antiviral therapy, while qualitative or quantitative HCV RNA detection is used to determine end-of-therapy and sustained virologic response to therapy.

The recombinant immunoblot assay (RIBA) has similar sensitivity to, but higher specificity than, screening tests, and was formerly used as a confirmatory test in patients with a positive screening antibody test for HCV. Patients with a positive screening test but negative RIBA results are considered not to have HCV infection (ie, falsely reactive screening test). Positive RIBA results (\geq two bands present) are indicative of chronic or resolved HCV infection, whereas those with a single band detected are considered indeterminate. Hepatitis C virus

Table XIV-16. Laboratory Diagnosis of Hepatitis C Virus (HCV)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
HCV IgG antibody (anti-HCV IgG) screen	Serum	Clot tube, RT, <2 h
HCV IgG antibody confirmation by recombinant immunoblot assay (anti-HCV RIBA)	Plasma	EDTA, RT, <2 h
HCV RNA detection, qualitative		
HCV RNA quantification (viral load)		
HCV genotyping		

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; RT, room temperature.

genotyping is used to guide the choice and duration of antiviral therapy and predict the likelihood of response to therapy, as different genotypes have varying susceptibilities to current treatment regimens.

A human genomic polymorphism interleukin-28B (IL-28B) genotype CC (within an interferon gamma promoter region), is associated with increased likelihood of sustained viral response in individuals with chronic hepatitis C virus infection undergoing treatment with pegylated interferon and ribavirin, and has strong predictive value for spontaneous resolution of infection.

The Centers for Disease Control and Prevention has recently recommended that adults born during 1945 and 1965 receive one-time testing for hepatitis C virus.

P. Enterovirus and Parechovirus

The enteroviruses that most often cause meningitis include certain echovirus and coxsackievirus serotypes and enteroviruses 70 and 71. NAAT of CSF is more sensitive than culture for the diagnosis of enteroviral central nervous system infection (Table XIV-17). Plasma or serum is useful for diagnosis of sepsis syndrome of the newborn due to enterovirus, but testing is less reliable outside of the newborn period. In the right clinical scenario, recovery of enterovirus from throat or stool may provide circumstantial etiologic evidence of central nervous system infection.

Serologic evaluation involves assessment of acute and convalescent titers, and is not typically useful in real-time clinical practice.

Parechoviruses have clinical presentations similar to enteroviruses, but are classified as a different genus and require a specific NAAT (laboratory validated only, no FDA-cleared tests) for detection.

Table XIV-17. Laboratory Diagnosis of Enteroviruses and Parechoviruses

Diagnostic Procedures	Optimum Specimen	Transport Issues; Optimal Transport Time
NAAT	Cerebrospinal fluid ^a	Sterile tube, RT, <2 h
	Serum	Clot tube, RT, <2 h
	Plasma (Blood is less reliable)	EDTA tube, RT, <2 h
	Urine	Sterile container, RT, <2 h
Culture	Throat	Sterile container or viral transport medium, RT, <2 h
	Stool	Sterile container, RT, <2 h
	Plasma (Blood is less reliable)	EDTA tube, RT, <2 h

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

^a A commercial FDA-cleared product is available for rapid PCR testing for enteroviruses in CSF.

¹A commercial FDA-cleared product is available for rapid PCR testing for enteroviruses in CSF.

Q. Respiratory Syncytial Virus

Respiratory syncytial virus causes bronchiolitis and/or pneumonia and is most common in infants and young children, although it can present in older individuals and cause severe disease in the immunocompromised. It is ideally detected by NAAT testing of secretions obtained by washing, suctioning, or swabbing the nasopharynx (Table XIV-18). Several FDA-cleared NAAT platforms exist. Culture is more time-consuming and less sensitive.

The presence of IgG generally indicates past exposure and immunity. The presence of IgM class antibodies or a 4-fold or greater rise in IgG titer between acute and convalescent sera suggests recent infection.

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 nasopharyngeal swabs (Table XIV-19). 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 may need to be confirmed by NAAT or culture. During seasons of low prevalence of influenza, false positive tests are more likely to occur with rapid screening procedures. Performance of influenza assays varies depending on the assay and the circulating strains. NAAT is now considered the gold

Table XIV-18. Laboratory Diagnosis of Respiratory Syncytial Virus (RSV)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
NAAT ^a	Nasopharyngeal aspirate/washing, throat or nasopharyngeal 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)	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT or ideally on wet ice, <2 h
Serology (IgM and IgG)	Serum	Clot tube, RT, <2 h

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; NAAT, nucleic acid amplification test; RT, room temperature.

^a Commercial products are available for rapid PCR testing for respiratory viruses.

standard for detection of influenza virus in clinical samples. Several FDA-cleared NAAT platforms exist.

Serologic evaluation involves assessment of acute and convalescent titers, but is not typically useful in real-time clinical practice.

Table XIV-19. Laboratory Diagnosis of Influenza A and B Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Rapid antigen detection	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT or ideally on wet ice, <2 h
Serology	Serum	Clot tube, RT, <2 h
	Cerebrospinal fluid	Sterile tube, RT, <2 h
NAAT ^a	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT, <2 h

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

^a Commercial products are available for rapid NAAT testing for respiratory viruses

Table XIV-20. Laboratory Diagnosis of West Nile Virus (and Eastern Equine, Western Equine, Saint Louis, and California Encephalitis Viruses)

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology	Serum	Clot tube, RT, <2 h
NAAT	Cerebrospinal fluid	Sterile tube, RT, <2 h
	Serum	Clot tube, RT, <2 h
	Plasma	EDTA tube, RT, <2 h

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

S. West Nile Virus

West Nile virus (and Eastern equine, Western equine, Saint Louis and California encephalitis viruses) cause central nervous system infections.

The laboratory diagnosis of West Nile virus is typically accomplished by detecting virus-specific IgM antibodies in serum (Table XIV-20). West Nile virus IgM antibodies may persist in serum for ≥ 6 months and false positive results may occur following recent yellow fever immunization or natural infection with other flaviviruses (eg, dengue, Saint Louis encephalitis). Acute (3–10 days after symptom onset) and convalescent (2–3 weeks later) serum for IgG serology may also be helpful. Positive antibody titers to West Nile virus are commonly present in older individuals, especially those from the Indian subcontinent (who presumably have been exposed to flaviviruses during their lifetimes). Therefore in patients where the pretest probability of infection with West Nile virus is low, the presence of West Nile virus antibodies in plasma or serum should be interpreted cautiously.

Serologic diagnosis of West Nile virus central nervous system infection is based on assessing the CSF to serum antibody index or the detection of West Nile virus IgM in cerebrospinal fluid. However, detection of antibody in cerebrospinal fluid may indicate central nervous system infection, blood contamination, or transfer of antibodies across the blood-brain barrier.

West Nile virus NAAT is insensitive in immunocompetent hosts, but more sensitive in immunocompromised hosts. Viremia typically drops to levels that may be undetectable by NAAT at the time of symptom onset. West Nile Virus NAAT testing is insensitive for central nervous system disease. Viral culture may be available in specialized laboratories but is also insensitive.

Eastern and Western equine, Saint Louis and California encephalitis virus infection may be diagnosed serologically following the same strategy used for West Nile virus.

T. Adenovirus

In otherwise healthy individuals, adenoviruses usually cause mild, self-limiting respiratory illnesses with most cases being

Table XIV-21. Laboratory Diagnosis of Adenovirus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
NAAT	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen, stool, conjunctiva swab, plasma, cerebrospinal fluid	Sterile container or viral transport medium, RT, <2 h
Rapid antigen detection	Nasopharyngeal swab, respiratory specimen	Sterile container or viral transport medium, RT, <2 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen, stool, cerebrospinal fluid	Sterile container or viral transport medium, RT, <2 h
Antigen detection (Adenovirus types 40 and 41)	Stool	Sterile container, RT, <2 h
Serology	Serum Cerebrospinal fluid	Clot tube, RT, <2 h Sterile tube, RT, <2 h

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

diagnosed on clinical grounds alone. Occasionally, adenovirus infections in immunocompetent hosts can be deadly, especially in children with asthma. In immunocompromised patients, adenoviruses may cause pneumonia, disseminated infection, gastroenteritis, hemorrhagic cystitis, meningoencephalitis, hepatitis, etc.

Diagnosis is based on NAAT, culture and/or compatible histopathology (Table XIV-21). Viral culture has a long turnaround time but is reduced if 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.

Serologic testing relies on demonstration of antibodies to group-specific antigens, and often requires analysis of acute and convalescent sera. Serologic diagnosis of central nervous system infection is based on CSF to serum antibody index, four-fold rise in acute to convalescent IgG titer, or a single positive IgM. Detection of antibody in CSF may indicate central nervous system infection, blood contamination, or transfer of antibodies across the blood-brain barrier.

U. Rabies Virus

Rabies virus infects the central nervous system and is most often transmitted through the bite of a rabid animal. State

Table XIV-22. Laboratory Diagnosis of Rabies Virus

Diagnostic Procedure	Optimum Specimen	Transport Issues; Optimal Transport Time
NAAT	Saliva	Sterile tube, RT, <2 h
Direct fluorescent antibody	Nuchal skin biopsy, brain	Sterile container, RT, <2 h
Serology	Serum Cerebrospinal fluid	Clot tube, RT, <2 h Sterile tube, RT, <2 h

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

Health Departments should be consulted immediately in cases of suspected rabies.

No single test is sufficient to diagnose rabies ante-mortem (Table XIV-22). Testing is performed on samples of saliva, serum, spinal fluid and skin biopsies of hair follicles at the nape of the neck. Saliva and CSF may be tested by culture and NAAT (laboratory-validated). Serum and CSF may be tested for antibodies to rabies virus. Skin biopsy specimens may be examined for rabies antigen in the cutaneous nerves at the base of hair follicles. Histopathologic evaluation and direct fluorescent antibody testing of brain biopsy material are helpful, if available.

Serologic testing may be used to document post-vaccination seroconversion in the immunocompromised, if there is significant deviation from a prophylaxis schedule or if an individual initiated treatment internationally with a non-cell culture vaccine.

V. Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis virus is a rodent-borne virus that can cause meningoencephalitis and may be life-threatening in immunosuppressed persons. Serologic diagnosis is based on a four-fold rise in acute to convalescent IgG titer, or a single positive IgM (Table XIV-23). Detection of antibody in CSF may indicate central nervous system infection, blood contamination, or transfer of antibodies across blood-brain barrier; CSF to serum antibody index may be helpful in interpreting CSF antibody results.

Table XIV-23. Laboratory Diagnosis of Lymphocytic Choriomeningitis Virus

Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Serology (IgG, IgM)	Serum Cerebrospinal fluid	Clot tube, RT, <2 h Sterile tube, RT, <2 h

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; RT, room temperature.

XV. BLOOD AND TISSUE PARASITE INFECTIONS

Blood and tissue parasites comprise a large number of protozoa and helminths found in tropical and temperate climates worldwide [238]. Certain parasites cause infections with associated high morbidity and mortality (eg malaria, amebic encephalitis) while others may cause 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 diagnosis of most blood and tissue parasitic infections [239, 240]. 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*, *Wuchereria*, and *Mansonella*, whereas microscopic examination and/or culture 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.

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 [239]. When available, antibody titers may be used to determine the strength of the immune response or detect a trend in antibody levels over time. Indirect fluorescent antibody assays (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 which are not entirely consistent with the clinical picture.

Laboratory methods that detect parasite antigens and/or DNA provide an attractive alternative to traditional morphologic and serologic techniques. For example, a simple rapid immunochromatographic card assay for the detection of *Plasmodium* has recently been approved by the FDA [241, 242]. It may find use in acute care settings such as emergency departments (EDs) or out-patient clinics to establish a diagnosis of malaria quickly while awaiting results of confirmatory blood films. This assay is adequately sensitive in typical patients with symptomatic malaria (“fever and chills”) but loses sensitivity if the parasitemia is very low or infection is due to non-*falciparum* species [241]. This is especially important in nonendemic settings such as the U.S. where patients often present with low parasitemia.

Finally, the Centers for Disease Control and Prevention (CDC) and a number of reference laboratories in the U. S. and Canada perform extremely sensitive nucleic acid detection methods 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, 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 evaluation if either agent is suspected.
-

Table XV-1. Laboratory Diagnosis of Blood and Tissue Parasitic Infections^{a,b}

Disease/Organism	Main Diagnostic Tests	Remarks
Amebic encephalitis due to <i>Naegleria fowleri</i> , <i>Acanthamoeba</i> spp, and <i>Balamuthia mandrillaris</i> (free-living amoebae)	Microscopy and culture of CSF or brain tissue PCR from unfixed tissue or CSF is available from the CDC. Stained and unstained tissue slides may also be sent.	Specimens for culture should not be refrigerated. <i>Balamuthia mandrillaris</i> does not grow on standard agar (requires specialized cell-culture).
Angiostrongyliasis and Gnathostomiasis	Serology from CDC or Faculty of Tropical Medicine, Mahidol University, Bangkok Thailand (http://www.tm.mahidol.ac.th/en/special)	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 <i>Babesia microti</i> , <i>B. divergens</i> , <i>B. duncani</i> and <i>Babesia</i> spp MO-1 strain	Microscopy of Giemsa stained thick and thin blood films Real time PCR available from CDC and reference labs.	Most commercially available NAAT assays detect <i>B. microti</i> only. Serology does not distinguish between acute and past infection.
<i>Baylisascaris</i> Encephalitis	Serology from the CDC Division of Parasitic Diseases, Parasite Serology Laboratory Larvae may be seen on histopathologic sections of brain tissue	Turnaround time can be long.
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).	Serology is confirmatory to radiologic and scan studies.
Filariasis due to species of <i>Wuchereria</i> , <i>Brugia</i> , and <i>Mansonella</i>	Microscopy of Giemsa stained thick and thin blood films. Examination of concentrated blood specimens (Knott's, Nuclepore filtered blood or buffy coat) increases sensitivity. Antibody and/or antigen detection EIA (<i>Wuchereria bancrofti</i> and <i>Brugia malayi</i>) in blood by the CDC or reference lab	Blood films for <i>W. bancrofti</i> and <i>B. malayi</i> should be collected at night when microfilariae are circulating. Repeat exams may be necessary due to low parasitemia. Serology does not differentiate between filariae.
Filariasis, onchocerciasis due to <i>Onchocerca volvulus</i>	Microscopy of "skin snip" after incubation in saline at 37°C [243]	"Skin snips" should be from areas near 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 available from reference laboratories; does not differentiate between filariae.
Leishmaniasis, cutaneous due to various <i>Leishmania</i> species	Microscopic exam of Giemsa stained smears of biopsy touch impressions or aspirate from leading edge of ulcer; culture may be available using special media (NNN and others) PCR and isoenzyme analysis are available at the CDC for speciation, which may be important for treatment considerations [244]	Histopathology of leading edge ulcer biopsies is less sensitive than impression smears. Serology is not useful for cutaneous disease.
Leishmaniasis, visceral, due to various <i>Leishmania</i> species	Microscopic exam of Giemsa stained bone marrow aspirate/biopsy, splenic aspirate; culture may be available using special media (NNN and others). Contact laboratory for availability of special media. Serology from the CDC or reference laboratory [244]	Positive rK39 serology reported to be both sensitive and specific for the diagnosis of visceral leishmaniasis in various endemic areas of the world.
Malaria due to <i>Plasmodium falciparum</i> , <i>P. ovale</i> , <i>P. vivax</i> , <i>P. malariae</i> , <i>P. knowlesi</i>	Microscopy of Giemsa stained thick and thin blood films (3 sets obtained during febrile episodes); antigen (HR-2, aldolase, pLDH) detection tests (BinaxNow is FDA approved in US) NAAT from some reference laboratories will detect and differentiate all species.	Antigen strip tests lack sensitivity in low parasitemia and non-<i>falciparum</i> malaria and do not differentiate all species.
Toxocariasis (visceral larva migrans)	Serology from CDC or referral laboratories Histopathology	Larvae may be seen in histopathologic sections of biopsies of liver or other infected tissues.

Table XV-1 continued.

Disease/Organism	Main Diagnostic Tests	Remarks
Toxoplasmosis due to <i>Toxoplasma gondii</i>	Serology (IFA, EIA, enzyme linked fluorescent assay) from CDC or reference laboratory for detection of IgM and IgG; Positive IgG seen in up to 15% to 40% of US population due to previous exposure. IgG avidity test and serial titers may distinguish between recent and past infection. NAAT is available from some reference labs.	Cysts and tachyzoites can be seen in specimens from immunocompromised patients (eg bronchoalveolar lavage, brain biopsy). Animal inoculation may be available from the CDC.
Trichinosis due to <i>Trichinella spiralis</i> and other species	Serology (EIA) from the CDC or reference laboratory [245] Histopathology	Encysted larvae can be seen in histopathologic sections of muscle biopsies.
Trypanosomiasis, African (Sleeping Sickness) due to <i>Trypanosoma brucei gambiense</i> (West African) or <i>T. b. rhodesiense</i> (East African)	Microscopy of Giemsa stained thick and thin blood films or buffy coat preps. Parasitemia is often low, requiring repeated exams. Centrifuged CSF may be examined but organisms are rarely seen. Aspirates of chancres and lymph nodes may also be examined. There is an infection hazard from live organisms in blood specimens. [246, 247]	Morula cells of Mott (plasma cells with large eosinophilic antibody globules) may be seen in CSF and brain biopsy. Card agglutination test for trypanosomiasis (CATT) is available in endemic settings for detection of <i>T. b. gambiense</i> infection. Contact the CDC or Parasite Diagnosis Unit, (Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium Phone: +32 3 247.66.66 - Fax: +32 3 216.14.31 - Email:info@itg.be (http://www.itg.be/itg/))
Trypanosomiasis, American (Chagas' Disease) due to <i>Trypanosoma cruzi</i>	Microscopy of Giemsa stained thick and thin blood films or buffy coat preps. Serology available for donor and diagnostic testing. Culture of blood may be available using special media (NNN and others). Contact the laboratory for availability of special media. There is an infection hazard from live organism in blood specimens [246, 248, 249].	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 and is different from the test used for diagnostic purposes.

Abbreviations: HRP2, histidine rich protein 2; IFA, immunofluorescence assay; NIH, National Institutes of Health; NAAT, nucleic acid amplification test; NNN, Novy-MacNeal-Nicolle medium; PCR, polymerase chain reaction.

^a "CDC" refers to the Division of Parasitic Diseases at the Centers for Disease Control and Prevention, Atlanta GA, (770) 488-4431. Central Telephone for the CDC: (404) 639-3311 and web: <http://www.cdc.gov/> or <http://www.dpd.cdc.gov/dpdx/>.

^b "Reference Laboratories" refers to any laboratory that performs esoteric testing not usually done in routine hospital labs; examples include Toxoplasma Serology Laboratory (<http://www.pamf.org/serology/>); 650-853-4828, ARUP (800) 522-2787, FOCUS Diagnostics ((703) 480-2500), and Mayo Medical Laboratories (800-533-1710). All have their own web sites.

- NAATs are useful for detection of low parasitemia or in specifically identifying organisms which cannot be differentiated microscopically.
- NAATs should not be used to monitor response to therapy, since DNA may be detectable for days to weeks after successful treatment.
- Nucleic acid detection of blood and tissue parasites is currently available only from specialized laboratories and turnaround time may be prolonged.

Table XV-1 presents an inclusive overview of the approach to the diagnosis of blood and tissue parasitic infections [238–240]. Important points are bolded. Subsequent sections A and

B provide more detailed information on the diagnosis of parasitic infections which 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 laboratories such as those of the CDC and World Health Organization (WHO) are more likely than commercial laboratories to have the experience and volume of specimens to properly validate the more esoteric tests, while turnaround time for results is often faster with commercial reference labs. Direct communication by phone or e-mail will sometimes hasten specimen processing and result reporting from public health

laboratories, especially when there is an urgent clinical situation. The DPDx website at CDC (<http://www.dpd.cdc.gov/dpdx/HTML/DiagnosticProcedures.htm>) provides a list of currently available diagnostic tests for parasitic infections available from the CDC. The CDC also provides a valuable consultation service that can be accessed through the DPDx website for both the laboratorian and clinician. The availability of rapid shipping methods (FedEx, UPS, etc.) and e-mail 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 U.S. and *B. divergens* in Europe. More recently, a small number of infections occurring in California and Washington have been attributed to *B. duncani*, while an unnamed species (MO-1 strain) has been detected from a fatal case in Missouri. Malaria is caused by *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*; the latter is primarily a simian parasite in Southeast Asia which has recently been recognized in an increasing number of human patients. Table XV-2 summarizes the laboratory tests available for these agents.

The standard method for diagnosis of both parasites is microscopic examination of Giemsa stained thick and thin blood films. Although this method requires a minimum amount of resources (staining materials and high quality microscopes), well trained and experienced technologists must be available to obtain maximum accuracy and efficiency [250]. Because both babesiosis and malaria are serious infections which can progress to fatal outcomes if not diagnosed and treated accurately, it is necessary for health care facilities to have ready access to rapid accurate laboratory testing. Ideally, samples are obtained from fresh capillary (or venous) blood and slides are prepared immediately. However, it is typically more practical to obtain EDTA (preferred) or heparin anticoagulated blood and transport the sample to the laboratory for slide preparation.

Thick blood films are essentially lysed concentrates which allow rapid detection of the presence of parasites consistent with either *Plasmodium* or *Babesia* but generally do not allow definitive identification. The thick film is made using 2–3 drops of blood that have been “laked” (lysed) by placement into a hypotonic staining solution. This releases the intracellular parasites and allows for examination of multiple (20–30) layers of blood simultaneously. For this reason, it is the most sensitive method for microscopic screening and allows detection of very low levels of parasitemia (less than 0.001% of RBCs infected). In contrast, the 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* speciation. It also allows for optimal evaluation and differentiation of malaria from *Babesia* parasites, although the different *Babesia* species 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.

Both thick and thin films should be screened manually, since automated hematology analyzers may fail to detect *Plasmodium* and *Babesia* species parasites. The slides should first be screened at low power (100 times final magnification) for identification of larger microfilariae, followed by examination under oil immersion. The laboratorian should examine a minimum of 300 microscopic fields at 500 to 1000 times total magnification on the thick and thin films before reporting a specimen as negative. 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 lab. In this situation, 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 or more specimens drawn during febrile episodes) are indicated if the initial film is negative, and malaria or babesiosis is strongly suspected.

When *Plasmodium* species are identified, one can enumerate the number of infected RBCs and divide by the total number of RBCs counted to arrive at the percent 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 may be used to guide initial treatment decisions and to follow a patient’s progress during treatment.

An alternative to Giemsa-stained blood films for morphologic examination is the Quantitative Buffy Coat (QBC) method. 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 U. S. 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. For these reasons, the QBC method is seldom used in the U.S. at this time.

Although morphologic examination is the conventional method for diagnosis of malaria, it requires considerable time and expertise. Rapid antigen detection tests (RDTs) for malaria

Table XV-2. Laboratory Diagnosis of Babesiosis and Malaria Infection

Diagnostic Procedures	Optimum Specimens	Transport Considerations	Estimated TAT ^a
Microscopy of Giemsa stained thick and thin blood films with determination of percent 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. If transport time is longer, thick and thin blood should be made at bedside but blood in EDTA tube may be refrigerated. Prolonged exposure to EDTA can alter parasite morphology. Thick blood films dry slowly and should be protected from inadvertent smearing or spillage and dust	2–4 h
Quantitative Buffy Coat Centrifugal (QBC) 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 or RDT)	Drop of blood from finger stick or venipuncture needle placed directly on RDT test pad	Test should be performed as soon as possible but blood may be stored at 2°–30°C for up to 3 d for some commercial assays	15–30 min
Serologic detection of antibody to <i>B. microti</i> and <i>Plasmodium</i> 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 if storage will be >48 h	1–2 h

Abbreviations: NAAT, nucleic acid amplification test; RBC, red blood cell; TAT, turnaround time.

^a Transportation time is not included in this estimate.

provide cost effective, rapid alternatives and can be used for screening when qualified technologists are not available. The BinaxNow rapid diagnostic test has recently been approved by the FDA. It is a rapid immunochromatographic card (or “dipstick”) assay which requires no specialized equipment or special training for qualified technologists. This RDT uses monoclonal antibodies to detect the HRP-2 antigen of *P. falciparum* and an aldolase common to all species of *Plasmodium*. Positive RDTs should be confirmed by examination of thick and thin blood films which are also necessary to determine which species other than *P. falciparum* (if the assay is aldolase positive but HRP-2 negative) is present and to determine the rate of parasitemia.

This RDT is somewhat less sensitive than a thick blood film and may be falsely negative in cases with very low rates of parasitemia. However, the sensitivity is comparable to blood smear in symptomatic malaria patients with *P. falciparum* infection. In addition, 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. The RDT should not be viewed as a replacement for blood films but rather as a substitute in situations where reliable blood films will not be readily available (off hours in the laboratory when skilled personnel are not available) 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 films.

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. Indirect immunofluorescent antibody (IFA) is the most readily available commercial assay for *Babesia* (Focus Diagnostics, Cypress CA,

and other reference laboratories). IgM titers $\geq 1:16$ and IgG titers $\geq 1:1024$ 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 suggests 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 CDC.

Rapid NAAT assays 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 are comparable in 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, thin

blood films must still be examined to determine the percent 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 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 or 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 XV-3). As with blood films for malaria and *Babesia*, a minimum amount of resources (staining materials and high quality microscopes), as well as proficient

Table XV-3. Laboratory Diagnosis of 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	Slides and wet preps should be made from blood within 1 h. If transport time is longer, blood films should be made at bedside but blood may be refrigerated.	2–4 h
	OR Buffy coat concentrate from anticoagulated venous blood in EDTA tube (thin smear or fresh wet prep for motile organisms)	Thick blood films dry slowly and should be protected from inadvertent smearing or spillage and dust.	
Microscopic examination of tissue aspirates/biopsies by Giemsa/hematoxylin & eosin (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 one hour of sampling. Tissues may require 1–2 d of fixation before staining and examination.	2 h–3 d
Culture in NNN or other suitable media with subsequent microscopic examination for motile trypanosomes. Contact laboratory for availability of special media.	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

Abbreviations: GI, gastrointestinal; TAT, turnaround time.

^a Turnaround time within laboratory; transportation time is not included in this estimate.

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 *T. 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 U.S. labs 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.

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 (NNN) or similar media of any appropriate blood or tissue specimen during the acute and chronic stages will add to the sensitivity of laboratory diagnosis. The laboratory should be contacted to assure the availability of special media. It must be emphasized that 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, since the infection has been shown to be transmitted by transfusion and transplantation. A somewhat unusual situation has developed for serologic testing for American trypanosomiasis where the FDA has approved two commercial assays for blood or organ donor screening and a different commercial assay for patient diagnostic testing. Each assay cannot be used for the nonapproved purpose even though they are supposed to be detecting the same antibodies. An ELISA (Ortho-Clinical Diagnostics, Raritan, NJ) and an automated method (Abbott Prism Chagas, Abbott Park, IL) have been approved for blood, organ, cell, and tissue donor screening whereas a different ELISA test (Hemagen Diagnostics, Columbia, Md) is approved for diagnostic testing. Donor screening test positives may be tested by an FDA approved supplemental test (ABBOTT ESA Chagas) and/or submitted to a reference laboratory for confirmatory testing by a radioimmuno-precipitation assay (RIPA). The Hemagen assay measures IgG and does not require confirmatory testing. Both ELISAs

provide only qualitative positive or negative results without information regarding antibody titer.

Notes

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