Clinical Infectious Diseases

IDSA GUIDELINES

Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2024 Update by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)*

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The critical nature of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician and the microbiologists who provide enormous value to the health care team. This document, developed by experts in both adult and pediatric laboratory and clinical medicine, provides information on which tests are valuable and in which contexts, and on tests that add little or no value for diagnostic decisions. Sections are divided into anatomic systems, including Bloodstream Infections and Infections of the

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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 arboviral Infections, Viral Syndromes, and Blood and Tissue Parasite Infections. Each section contains introductory concepts, a summary of key points, and detailed tables that list suspected agents; the most reliable tests to order; the samples (and volumes) to collect in order of preference; specimen transport devices, procedures, times, and temperatures; and detailed notes on specific issues regarding the test methods, such as when tests are likely to require a specialized laboratory or have prolonged turnaround times. In addition, the pediatric needs of specimen management are also addressed. 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.

I. EXECUTIVE SUMMARY

INTRODUCTION

Diagnostic stewardship of infectious diseases focuses on optimizing patient care and outcomes utilizing a team approach comprised of highly skilled partners including clinicians, nurses, laboratorians, and other medical staff [1]. One key component of infectious disease diagnosis is the microbiology laboratory staffed by experienced clinical microbiologists. 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 quickly adapt to environments where antibiotics and host responses apply pressures that encourage their survival. A laboratory instrument may or may not detect those mutations or the genes that lead to them, so a certified specialist in microbiology is needed to facilitate microbiology laboratory result interpretation and to incorporate the clinical relevance of the results. Clearly, microbes grow, multiply, and die very quickly. If any of those events occur during the preanalytical phase, which includes all steps prior to testing (i.e., test ordering, specimen selection, collection, transport and 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 and relevance and is usually not under the direct control of the clinical microbiologist.

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 for laboratories. To provide that level of quality and accurate results, the laboratory requires that all

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microbiology specimens be properly and carefully selected, collected, and transported to optimize analysis and interpretation. Because accurate result interpretation in microbiology depends entirely on the quality of the specimen submitted for analysis, specimen management cannot be left to chance, and those that collect specimens for microbiologic analysis must be aware of what the physician needs for patient care as well as what the laboratory needs to optimize results, including ensuring that specimens arrive at the laboratory for analysis as quickly as possible after collection [2] (Table 1). In the tables throughout the document, when "immediately" is used in the Transport Issues column, the implication is to get the specimen to the laboratory without delay after collection.

Microbial names continue to change because of more sophisticated methods of genetic analysis and taxonomic flexibility. While the changes in nomenclature may be initially confusing, those changes may clarify clinical correlations that were previously obscured and could also impact antimicrobial susceptibility profiles that were initially confusing. We refer readers to the following sources for current taxonomy: Bacteria: www.bacterio.net/-alintro.html;

Fungi: www.mycobank.org ; Viruses: http://ictvonline.org; Parasites: www.cdc.gov/dpdx. Other sources are also available.

Table 1. TRANSPORT ISSUES (general guide) *

Specimen Type	Specimen Required	Collection Device, Temperature, and Ideal Transport Time
Aerobic bacterial culture	Tissue, fluid, aspirate biopsy, etc.	Sterile container, Room temperature (RT), immediately
	Swab (2nd choice) – flocked swabs are recommended over spun swabs	Swab transport device, RT, 2h
Aerobic and anaerobic bacterial culture	Tissue, fluid, aspirate, biopsy, etc.	Sterile anaerobic container, RT, immediately
	Swab (2nd choice) – flocked swabs are effective	Anaerobic swab transport device, RT, 2h
Fungus culture; AFB culture	Tissue, fluid, aspirate, biopsy, etc.	Sterile container, RT, 2h
	Swab (2nd choice) (for yeast and superficial mycobacterial infections only)	Swab transport device, RT, 2h
Virus culture	Tissue, fluid, aspirate, biopsy, etc.	Viral transport media, on ice, immediately
	Swab – flocked swabs are recommended over spun swabs	Virus swab transport device, RT, 2h
Suspected agent of bioterrorism	Refer to Centers for Disease Control and Prevention website for specimen collection and shipping: https://emergency.cdc.gov/labissues/index.asp Guidance on the Transfer of Select Agents and Toxins | Compliance | Federal Select Agent Program	
Serology	1-5 ml serum depending on test used	Clot tube, RT, 2h
Antigen test	As described in the laboratory specimen collection manual	Closed container, RT, 2h
NAAT	5 ml plasma-follow manufacturer recommendations	EDTA tube, RT, 2h
	Other specimen, i.e. viral transport medium	Closed container, RT, 2h

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*RT (Room Temperature); 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. The 2h transport time in this and subsequent tables in the document emphasizes the optimum time between collection and culture. Specimens placed into appropriate transport media will maintain viability of organisms beyond 2h but in all cases, the manufacturer's instructions should be followed. Consultation with the laboratory is recommended.

At an elementary level, the physician needs answers to three very basic questions from the laboratory: 1) Is my patient's illness caused by a microorganism? 2) If so, what is it? 3)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 laboratory for analysis. Caught in the middle, between the physician and laboratory, are the medical staff who have received orders to 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 patient-facing healthcare provider and laboratory staff should be encouraged and open with no punitive motive or consequences.

The diagnosis of infectious disease is best achieved by applying in-depth knowledge of both medical and laboratory science along with principles of epidemiology and pharmacokinetics of antibiotics and by integrating a strategic view of host-parasite interactions. Clearly, the best outcomes for patients are the result of strong partnerships between the clinician and the microbiology specialist. This document illustrates and promotes this partnership and emphasizes the importance of appropriate specimen management to clinical relevance of the results. One of the most valuable laboratory partners in infectious disease diagnosis is the microbiology 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. These Board-Certified microbiology specialists are engaged with the medical staff to communicate clinically significant results, interpret complex laboratory findings, and consult on testing and diagnostics on a daily basis. 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 expert 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 helps drive hospital infection control, it influences patient length of stay, hospital costs, and laboratory costs, it impacts antibiotic stewardship, and

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influences laboratory efficiency. Clinicians and medical staff should consult the laboratory to ensure that selection, collection, transport, and storage of the patient specimens they collect are managed properly.

Antibiotic stewardship and diagnostic stewardship are interdependent and the laboratory plays a pivotal role in each of those activities. Diagnostic stewardship leads to the appropriate use of laboratory testing to guide clinical intervention and therapeutics in order to optimize patient outcomes and limit the spread of antimicrobial resistance (which is addressed by antibiotic stewardship) [1]. The importance and interdependence of antibiotic and diagnostic stewardship are shown in Figure 1. This document focuses on diagnostic stewardship and how best to optimize the use of the microbiology laboratory for infectious disease diagnosis.

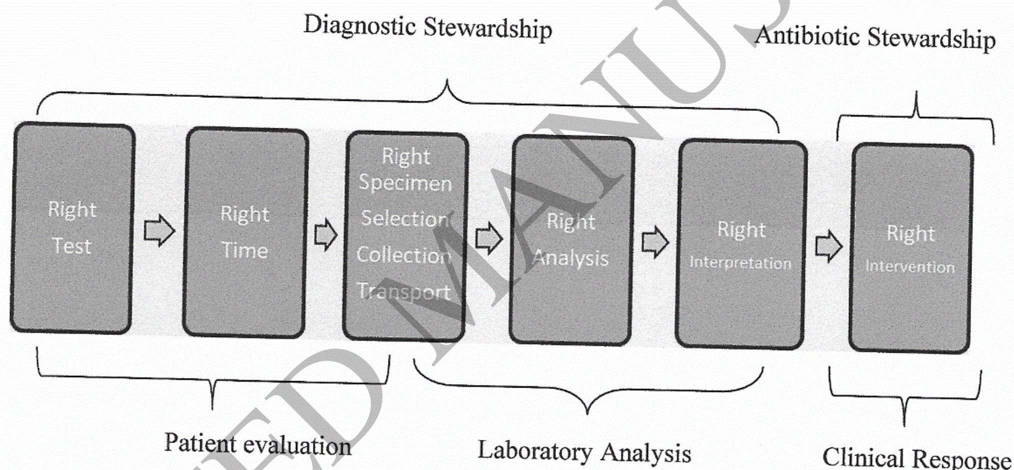

Figure 1. Interaction of diagnostic stewardship and antibiotic stewardship resulting in positive patient outcomes.

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 that do not meet the requirements specified in the lab procedure manual.

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2. Full identification of multiple organisms (>3) within a mixed culture is not recommended. This can provide irrelevant information that could result in inaccurate diagnosis and inappropriate therapy and place the laboratory at legal risk for providing such information.

3. "Background noise" of commensal microbiota unrelated to the disease process must be avoided where possible during specimen collection. Many body sites have normal microbiota that can easily contaminate the specimen and complicate interpretation. Therefore, specimens from sites such as lower respiratory tract (sputum), nasal sinuses, superficial wounds, fistulae, and others that contain large numbers of normal microbiota require care in collection and interpretation.

4. The laboratory needs a specimen, not a swab of a specimen. Actual tissue, aspirates, and fluids are always preferred over swabs, especially from surgery. A swab is not the specimen of choice for many specimens because swabs pick up extraneous microbes as opposed to needle aspirates into a targeted area, 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. While nasal washes and nasopharyngeal aspirates might be the specimens of choice for viral and respiratory specimens, swabs are now commonly used. Flocked swabs have become a valuable tool for specimen collection and have been shown to be more effective than Dacron, rayon, and cotton wrapped swabs. 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 supported by the literature. Occasionally, clinicians will need the laboratory to run tests that are not FDA cleared, a similar scenario that drugs that are not FDA approved are often used. There is a difference between these two FDA categories, i.e., it is against the law for the lab to run a test that is not FDA cleared (or validated in house). It is not against the law for a physician to use a drug off label.

6. When possible a specimen should be collected prior to administration of antibiotics. Once antibiotics have been started, the microbiota changes and etiologic agents are affected, leading to potentially misleading culture results.

7. Susceptibility testing should be done only on clinically significant isolates, not necessarily on all microorganisms recovered in culture.

8. Microbiology laboratory results that are reported should be accurate, significant, and clinically relevant.

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9. The laboratory should set technical policy; this is not the purview of the medical staff. Good communication and mutual respect will lead to collaborative policies.

10. Specimens must be labeled accurately and completely so that interpretation of results will be reliable. Labels such as "eye" and "wound" are not helpful to the interpretation of results without more specific site and clinical information (for example: dog bite wound right forefinger).

Addressing swabs as a specimen collection method – to swab or not to swab!

Nothing is more important than the correct specimen source, adequate specimen volume, and condition of the specimen submitted for analysis. If the laboratory receives a specimen that was improperly selected, collected, or transported, the lab can contribute little or nothing to any investigation or to the welfare and outcome of the patient and may provide misleading information.

With few exceptions, swabs may be either acceptable or unacceptable for the best microbiology results based on specimen source. It is critical to know the difference! Although a swab may be the specimen of choice for some specimens, for many it is suboptimal for the laboratory to receive. While tissue and fluids are the specimens of choice when considering anaerobic bacteria, the use of flocked swabs in transport medium has been shown to be effective. In fact, flocked swabs and sponge-tipped swabs have several advantages over the classic swab wound with cotton or Dacron. Although minor differences may be seen in this document, a general rule of thumb about selecting and using a swab for specimen collection is described below.

Always use a swab as the preferred (or manufacturer designated) collection device for – throats, conjunctiva, superficial skin lesions for aerobic culture only, many microbial screening tests, some nose and nasopharynx specimens, some vaginal specimens, rectal specimens for proctitis, and upon recommendation of the manufacturer of an FDA-cleared test or local laboratory recommendation,

Sometimes, reluctantly, use a swab – open wound, pus (only if accompanied by a sample of the advancing margin of the wound; needle aspirate preferred), nasopharynx if wash/aspirate cannot be obtained. Strongly resist swabs in surgery and opt for tissue and fluid (inflamed tissues and spaces can be irrigated with nonbacteriostatic saline and the aspirate submitted).

Never use a swab – surgical tissue, from surgical field if tissue can be submitted, any body fluid, respiratory fluids and secretions, endophthalmitis and keratitis, nasal sinus, otitis media, biopsy, abscess fluid, fungal and acid-fast bacilli specimens, formed stool, epiglottitis, diarrheal illness, and when anaerobes are suspected opt for tissue or fluid in anaerobic transport (see flocked swab statement above). Never submit a swab for analysis that has been dipped into a fluid or exudate. Send an adequate volume of the fluid or exudate instead.

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It would be prudent for Infectious Diseases Service, Surgical Service, Infection Prevention, and Laboratory to address and resolve these issues and to clarify the needs of the clinician and the needs of the microbiology laboratory. Call the microbiology laboratory if there is a question of whether to use a swab or what type of swab should be used.

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 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 are tables 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. There are some instances where the listed procedures are essentially equal in their utility and thus cannot be distinguished in preferability.

Common abbreviations used throughout the text:

CDC, Centers for Disease Control and Prevention; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; DNA, deoxyribonucleic acid; EIA, enzyme immunoassay; FDA, Food and Drug Administration; GI, gastrointestinal; IFA, indirect fluorescent antibody; IIF, indirect immunofluorescence; MALDI-TOF-MS, matrix- assisted laser desorption/ionization-time of flight mass spectrometry; mpox, refers to the disease caused by Monkeypox virus; MRSA, methicillin-resistant *Staphylococcus aureus*; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction; PMN, polymorphonuclear neutrophil; RPR, rapid plasma reagin (test for syphilis); RNA, ribonucleic acid; RT, room temperature; VRE, vancomycin-resistant enterococcus; WBC, white blood cell; WHO, World Health Organization;

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When room temperature (RT) is specified for a certain time period, such as 2h, 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 >48h, although specimens in viral transport media may be transported at room temperature when rapid (<2h) delivery to the laboratory is assured.

History and Update

The first publication of this document appeared in 2013 followed by an updated version in 2018. The current 2024 version provides new knowledge, discusses new infections, and suggests new laboratory procedures to assist in confirming the causes of infectious diseases. Since the 2018 publication, we have experienced a Coronavirus pandemic that impacted the way clinicians evaluated their patients as well as how the laboratory approached diagnostic methods in the face of resource, personnel, and supply challenges. We have witnessed the emergence of Monkeypox outbreaks, new tickborne infections, the continuing emergence of *Candida auris* and other common and uncommon infectious agents requiring serologic or molecular approaches in laboratory testing as well as astute clinical diagnosis and intervention. Further, the emergence of complex microbial resistance mechanisms continue to complicate clinical and laboratory contributions. As a result, the literature has exploded with new data that needs to be incorporated.

The purpose of these guides was two-fold: 1) To ensure that clinicians fully understood the unique needs and services of the microbiology laboratory in support of their clinical diagnoses and, 2) to make laboratorians aware of the medical implications of serious infections in patients in support of clinical interventions. It is this approach and in-depth understanding of both clinical and laboratory issues that make this document unique and valuable to both physicians and laboratorians".

This document has been endorsed by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). This is not an official guideline of the IDSA but rather an authoritative guide with recommendations for utilizing the microbiology laboratory in infectious disease diagnosis. It is a collaborative effort between clinicians and laboratory experts focusing on optimum use of the laboratory for positive patient outcomes. When the term "recommended" is used in this document, it is not a "graded" recommendation as would be found in a guideline, but rather the preferred or indicated approach for use or application. Future modifications are to be expected, as diagnostic microbiology is a dynamic and rapidly changing discipline. While pediatric concerns have been integrated into the appropriate sections in this document, readers are referred to Pediatric Clinical Practice Guidelines & Policies, 19th edition, and The Red Book (2021) published by the American Academy of Pediatrics for detailed approaches in pediatric care.

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II. Bloodstream infections and infections of the cardiovascular system

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 24-48 h; incubation for more than 5 days seldom is required when modern automated continuous-monitoring blood culture systems and media are used [3, 4]. This includes recovery of fastidious organisms such as HACEK (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella,* and *Kingella*) bacteria and *Brucella* spp [5, 6]. Several *Haemophilus* species have been reclassified under the genus *Aggregatibacter*. Some microorganisms, such as *Cutibacterium acnes*, mycobacteria and dimorphic fungi, require longer incubation periods and special culture media or non-culture-based methods (DOI: 10.1016/j.cmi.2016.12.026). Although filamentous fungi often require special broth media or special specimen processing (such as lysis-centrifugation) for detection, most *Candida* spp grow in standard blood culture broths unless the patient has been on antifungal therapy. Unfortunately, blood cultures from patients with suspected candidemia do not yield positive results in almost half of patients. Table 2 provides a summary of diagnostic methods for most BSIs.

Blood culture collection. For most etiologic agents of infective endocarditis, conventional blood culture methods will suffice [5-7]. However, some less common etiologic agents cannot be detected with current blood culture methods. *Bartonella* spp and *Coxiella burnetii*, often can be diagnosed by conventional serologic testing [8, 9]. However, molecular amplification methods may be needed for detection of these organisms as well as others (e.g., *Tropheryma whipplei*). In rare instances of culture-negative endocarditis, 16S PCR and DNA sequencing as well as culture of valve tissue may help determine an etiologic agent.

The volume of blood that is obtained for each blood culture (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 [3, 4, 7, 10, 11]. For adults, 20-30 ml of blood per culture set is recommended and may require inoculation of more than two culture bottles depending on the system [4] . Both aerobic and anaerobic bottles should be inoculated. For children, an age- and weight- appropriate volume of blood should be cultured (Table 3). 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, two (or sometimes three) blood cultures (i.e. culture sets) should be submitted to the laboratory for the evaluation of each septic episode, with the most important consideration for obtaining more than two cultures being the volume of blood sampled [7, 12].

There is typically no need to space collection of blood cultures and so the two cultures should be collected by separate venipunctures, one after the other.

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Contaminated blood culture bottles are common, costly to the health care system, and frequently confusing to clinicians. To minimize the risk of contamination of the blood culture with commensal skin microbiota, meticulous care should be taken in skin preparation prior to venipuncture. In addition, products are available that allow diversion and discard of the first few milliliters of blood that are most likely to contain skin contaminants. Consensus guidelines [4] and expert panels [3] 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 [3, 4]. Iodine tincture and CHG require about 30 seconds to exert an antiseptic effect compared with 1.5-2 min. for povidone-iodine preparations [3]. Two recent studies have documented equivalent contamination rates with iodine tincture and CHG [13, 14]. CHG is not recommended for use in infants under 2 months of age, but povidone-iodine followed by alcohol is recommended.

Blood cultures contaminated with skin microbiota during collection are common, but laboratories should be able to achieve contamination rates below 3% with target rates of 1% when best practices are used [4]. Laboratories should have policies and procedures for abbreviating the work-up and reporting of common blood culture contaminants (e.g., coagulase-negative staphylococci, 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 be notified in real time (e.g., phone, auto-page) by the laboratory when a blood culture becomes positive since BSI's often represent life-threatening events. 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.

Nucleic acid–based tests offer multiplexed detections identifying the most common bacteria and fungi isolated from blood cultures. Many of these tests can also detect antimicrobial resistance markers and/or perform phenotypic AST. Results should be interpreted in conjunction with the detected organism. Other non–nucleic acid–based methods (eg, MALDI-TOF MS) have been used to identify bacteria and fungi directly from positive blood cultures. These tests identify the organism within one to three hours [4].

Key points for the laboratory diagnosis of bloodstream infections

- Volume of blood collected, not timing, is most critical
- Disinfect the venipuncture site with chlorhexidine or 2% iodine tincture in adults and children >2 mo old (chlorhexidine NOT recommended for children <2 mo old
- Draw blood for culture before initiating antimicrobial therapy

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- Catheter-drawn blood cultures have a higher risk of contamination (false positives)
- Do not submit catheter tips for culture
- 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 better in anaerobic than aerobic bottles

Table 2. Summary of diagnostic methods for most blood stream infections

Etiologic agents:	Diagnostic procedures:	Optimum specimens:	Transport issues
Staphylococcus spp *Streptococcus* spp *Enterococcus* spp *Listeria monocytogenes* *Enterobacterales* *Pseudomonas* spp *Acinetobacter* spp HACEK[a] bacteria *Brucella* spp anaerobic bacteria	Adults: 2-4 blood cultures Infants & children: 2 or more blood cultures	20-30 ml of blood per culture set in adults evenly distributed between 2 (or more) blood culture vials[b] Blood volume depends on the child's weight (see Table 3)[c]	Inoculated culture vials should be transported to the lab ASAP at RT; organisms will usually survive in inoculated culture vials even if not incubated immediately
Bartonella spp	2 or more lysis-centrifugation (Isolator™) blood cultures[d,e] (Currently not available in the U.S.) NAAT Serology for IgM/IgG	10 ml of blood should be inoculated directly into each lysis-centrifugation culture vial (Currently not available in the U.S.) 5ml of plasma Serology for IgM/IgG	Lysis-centrifugation culture vials[e] should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation (Currently not available in the U.S.) EDTA tube, RT, 2h Serology for IgM/IgG
Legionella spp	2 or more lysis-centrifugation (Isolator™) blood cultures[e,f] (Currently not available in the U.S.) *Legionella* urine antigen test (for Serotype 1)	10 ml of blood should be inoculated directly into each lysis-centrifugation culture vial[e] (Currently not available in the U.S.)	Lysis-centrifugation culture vials[e] should be transported at RT to the laboratory ASAP and processed within 8 h of blood inoculation

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Coxiella burnetii *Tropheryma whipplei*	Coxiella IFA serology NAAT NAAT	10 ml of mid-stream clean catch urine[g] 5 ml of serum 5 ml of plasma 5 ml of plasma	(currently not available in the U.S.) Closed container, RT, 2h Clot tube, RT, 2 h EDTA tube, RT, 2 h EDTA tube, RT, 2h
Yeast Filamentous and dimorphic fungi[i]	2-4 blood cultures in adults (see above) 2 or more blood cultures in infants and children (see above)	20-30 ml of blood per culture in adults evenly distributed between 2 blood culture vials[h] Maximum volume safely obtained from children; volume depends on weight of child (see following Table)[c]	Inoculated culture vials should be transported at RT to the lab ASAP for early incubation Inoculated vials for direct detection of *Candida* spp by T2 magnetic resonance assay may be used [15] Organisms will usually survive in inoculated culture vials even if not incubated immediately
Mycobacteria	3 cultures using AFB-specific blood culture vials	5 ml of blood inoculated directly into AFB-specific blood culture vial	Inoculated culture vials should be transported to the lab ASAP for early incubation

[a]HACEK bacteria include Haemophilus parainfluenzae, Aggregatibacter (Haemophilus) aphrophilus, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens and Kingella kingae.
[b]Typically, blood specimens are split between aerobic and anaerobic blood culture vials. There may be special circumstances in which omitting the anaerobic vial and splitting blood between aerobic vials is acceptable (e.g., fungemia due to yeast suspected).

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[c]Use pediatric blood bottles or routine aerobic bottles depending on volume of blood collected and manufacturer's instructions. Table 3 shows pediatric blood volumes. When 10 ml of blood or less is collected, it should be inoculated into a single aerobic blood culture vial.
[d]The recovery of Bartonella spp from blood even when optimum methods are used is extremely low and not recommended as a routine option.
[e]Lysis centrifugation tubes are not available commercially in the United States at the time of this writing. Future commercial availability is not known.
[f]Legionella bacteremia occurs very infrequently and rarely is the organism recovered from blood even when optimum culture techniques are employed.
[g]The optimum urine specimen is the first voided specimen of the day.
[h]Since yeast are highly aerobic, when fungemia due to yeast is suspected, it might be prudent within a series of blood cultures, to inoculate at least one blood specimen into two aerobic vials rather than the customary aerobic and anaerobic vial pair.
[i]Some dimorphic fungi and yeasts (e.g. Malassezia spp) may be visualized on peripheral blood smears in some patients using a variety of fungal stains.

Table 3. Recommended volumes of blood for culture in pediatric patients [3, 8]:

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 No. 1	Culture 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 or less

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 [15-19]. 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 [19].

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 [20]. Methods described include the following:

- Standard blood cultures (BCs), 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

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than 2 h before the BC drawn by venipuncture, there is a high probability of catheter-associated BSI [16, 21].

- Catheter tip or segment cultures. The semiquantitative method of Maki et al [18] is used most commonly but 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 over agar on a 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. Biofilm formation on catheter tips prevents antimicrobial therapy to clear agents within the biofilm thus requiring removal of the catheter to eliminate the organisms.
  Routine culture of IV catheter tips at the time of catheter removal has no diagnostic value and should not be done.

Infected (mycotic) aneurysms and vascular grafts

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

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

Etiologic agents	Diagnostic procedures	Optimum specimens	Transport issues
Bacteria	Gram stain Aerobic bacterial culture[a] Blood cultures (see I-A above)	Lesion biopsy or resected graft material[b]	Sterile container, RT, immediately
Fungi	Calcofluor-KOH stain Fungal culture Blood cultures (see I-A above)	Lesion biopsy or resected graft material[b]	Sterile container, RT, 2 h

[a]If aerobic bacteria are suspected. If anaerobes are suspected, then the culture should consist of an aerobic and anaerobic bacterial culture.
[b]Swab specimens of infected sites, even when collected using sterile technique during surgery, are always inferior to tissue specimens or a portion of the graft material.

Pericarditis and myocarditis

Numerous viruses, bacteria, rickettsia, fungi, and parasites have been implicated as etiologic agents of pericarditis and myocarditis. In many patients with pericarditis and in the overwhelming

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majority of patients with myocarditis, an etiologic diagnosis is never made and patients are treated empirically. In selected instances, however, a microbiologic diagnosis should be pursued aggressively as it is important clinically to define the specific cause of infection (Table 5). Some of the more common and clinically important pathogens will be reviewed in the tables below. When a microbiologic diagnosis of less common etiologic agents is required, especially when specialized techniques or methods may be necessary (e.g., 16S ribosomal PCR), consultation with the laboratory director should be undertaken. There is considerable overlap between pericarditis and myocarditis (addressed below) with respect to both etiologic agents and disease manifestations.

Table 5. Laboratory Diagnosis of Pericarditis and Myocarditis

Etiologic agents[a]	**Diagnostic procedures**	**Optimum specimens**	**Transport issues**
Bacteria	Gram stain Aerobic bacterial culture[b] Blood cultures (see I-A above)	Pericardial fluid or pericardial biopsy	Sterile container, RT, immediately
Fungi	Calcofluor-KOH stain Fungal culture Blood cultures (see I-A above)	Pericardial fluid or pericardial biopsy	Sterile container, RT, 2 h
Mycobacteria	Acid fast smear AFB culture Blood cultures (see I-A above)	Pericardial fluid or pericardial biopsy[c]	Sterile container, RT, 2 h
Coxsackie B virus Coxsackie A virus Echovirus Polio virus Adenovirus Mumps virus Cytomegalovirus Other viruses	Virus-specific serology Virus-specific NAAT Virus culture Histopathologic examination	Acute and convalescent sera Pericardial fluid or pericardial biopsy Pericardial fluid or pericardial biopsy Pericardial fluid or pericardial biopsy	Clot tube, RT, 2h Closed container, RT, 2 h Virus transport device, on ice, immediately Place in formalin and transport to histopathology lab for processing
Trypanosoma cruzi, T. b. gambiense	Parasite-specific serology	Acute and convalescent sera	Clot tube, RT, 2h

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T. b. rhodesiense *Trichinella spiralis* *Toxoplasma gondii* *Toxicara canis*	Blood smears[d] Histopathologic examination	5 ml of peripheral blood in an EDTA tube Endomyocardial biopsy or surgical specimen	Consultation with the laboratory is recommended Place in formalin and transport to histopathology lab processing

[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, Schistosoma, and Mycoplasma spp

[b]If anaerobic bacteria 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]Blood smears may be useful in detection infection caused by Trypanosoma spp

III. 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, three or four tubes of cerebrospinal fluid (CSF) are collected by lumbar puncture for diagnostic studies. The first tube has the highest potential for contamination with skin microbes and should not be sent to the microbiology laboratory for direct smears, culture, or molecular studies. A minimum of 0.5-1 mL of CSF should be sent immediately after collection to the microbiology laboratory in a sterile container for bacterial Gram stain and culture 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 by the clinician. 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 called to the patient care area immediately. Identification and susceptibility testing of bacteria recovered from cultures is routinely performed unless contamination during collection or processing is suspected.

Most clinical microbiology laboratories do not perform all of the testing listed in the tables. This is especially true of serologic and many molecular diagnostic tests. The availability of FDA-cleared NAATs for many agents is limited, requiring laboratory-developed tests to be used, with variable sensitivities and specificities. NAATs designed to detect a single target may be more

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sensitive than a multiplex assay. An FDA-cleared multiplex PCR (BioFire FilmArray [FA]) targeting 14 organisms for diagnosing meningitis and encephalitis (FA-ME) is only an adjunct to culture and other conventional test methods since false negatives (e.g., *Cryptococcus*) and false positives (e.g., *S. pneumoniae, S. agalactiae, H. influenzae*) occur [22-26]. Two systematic review and meta-analyses of the FA-ME noted suboptimal detection of some targets (e.g., *Listeria monocytogenes, H. influenzae, Escherichia coli*, HSV) and discuss how testing for low prevalence disease contributes to false positive results [27, 28]. Caution in interpreting syndromic panel results is especially needed for latent viruses like HHV-6 that usually lack clinical significance unless the patient is immunocompromised with imaging and laboratory findings to support the diagnosis [29, 30]. The decision to implement a syndromic panel, which patients to test, and how to confirm results continue to be topics of debate that should be discussed with relevant stakeholders at each institution [31-33]. The expense and data interpretation challenges associated with next generation sequencing have limited use of this technology to serious infections after the most common etiologies have been ruled out [34]. In clinical scenarios where serology is helpful, diagnosis may be based on CSF to serum antibody index, 4-fold rise in acute to convalescent IgG titer, or a single positive 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 d 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 culture sets should also be obtained if bacterial meningitis is suspected
- Alert the microbiology laboratory if unusual organisms are suspected (e.g., prion, *Nocardia spp*, *Mycoplasma spp*), for which special procedures are necessary
- Do not refrigerate cerebrospinal fluid
- CSF tubes #2 or #3, NOT #1 should be submitted for bacterial culture and molecular testing
- Attempt to collect as much sample as possible for multiple studies (minimum recommended is 1 ml); prioritize multiple test requests on small volume samples

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Meningitis

The most common etiologic agents of acute meningitis are viruses (enteroviruses and parechoviruses) and bacteria (*S. pneumoniae* and *N. meningitidis)* (Table 6). Patient age and other factors (i.e., immune status, post neurosurgery, trauma) are associated with specific pathogens.

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Molecular testing has replaced viral culture for the diagnosis of enteroviral meningitis, but is not routinely relied on for the detection of bacteria in CSF (Gram stain and bacterial culture should be ordered). The sensitivity of the Gram stain for the diagnosis of bacterial meningitis is 60-80% in patients who have not received antimicrobial therapy and 40-60% in patients who have received treatment [35]. Bacterial antigen testing on CSF is no longer recommended and should not be ordered nor should the laboratory provide this service. Early, incorrect assumptions held that selected antigen tests on CSF may have some value in patients who received therapy prior to specimen collection with negative Gram stain and negative culture results [36], but this is no longer recommended. In patients suspected of having bacterial meningitis, at least 2-4 blood culture sets should also be performed but therapy should not be delayed.

Organisms expected to cause chronic meningitis (symptoms lasting ≥4 weeks) include *M. tuberculosis*, fungi, and spirochetes (Table 6). Because the sensitivity of nucleic acid amplification tests (NAAT) for *M. tuberculosis* in nonrespiratory specimens may be poor, culture should also be requested [37, 38]. The reported sensitivity of culture for diagnosing tuberculous meningitis is 25-70% [39]. 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 [a]. Testing of CSF for anti-*Coccidioides* antibodies and *Coccidioides* antigen is recommended for the diagnosis of coccidioidal meningitis since direct fungal smear and culture are often negative [b].

Table 6. Laboratory Diagnosis of Meningitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			
Streptococcus pneumoniae *Neisseria meningitidis* *Listeria monocytogenes* *Streptococcus agalactiae* *Haemophilus influenzae* *Escherichia coli* *Citrobacter koseri (diversus)* Other Enterobacterales *Elizabethkingia meningoseptica*	Gram stain[1] Aerobic bacterial culture	Cerebrospinal fluid	Sterile container, RT, immediately
	Blood cultures	Blood, 2-4 sets	Blood culture bottles, RT, 2 h

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Mycobacterium tuberculosis	AFB smear AFB culture	Cerebrospinal fluid (≥5 mL)	Sterile container, RT, 2 h
	M. tuberculosis NAAT[2]	Cerebrospinal fluid	Sterile container, RT, 2 h
Spirochetal			
Treponema pallidum (syphilis)	VDRL, FTA-ABS	Cerebrospinal fluid	Sterile container, RT, 2 h
	Traditional: RPR screening test with positive RPR confirmed by *T. pallidum* particle agglutination (TP-PA) test or other treponemal confirmatory test Reverse sequence: EIA or chemiluminescent immunoassay treponemal screening test with positive confirmed by RPR (negative RPR reflexed to TP-PA)	Serum	Clot tube, RT, 2 h
Borrelia burgdorferi (Lyme disease)	*B. burgdorferi* antibodies, IgM and IgG with Western blot assay confirmation[3]	Serum	Clot tube, RT, 2 h
		Cerebrospinal fluid	Closed container, RT, 2 h
	B. burgdorferi NAAT (low sensitivity)	Cerebrospinal fluid	Sterile container, RT, 2 h
Leptospira spp	*Leptospira* NAAT[4]	Blood	EDTA or sodium citrate tube, RT, 2 h
		Cerebrospinal fluid Urine	Sterile container, RT, 2 h
	Leptospira culture (special media required; rarely available in routine laboratories)	1st week of illness: Cerebrospinal fluid, 10 mL blood	Sterile container, heparin or citrate tube, RT, immediately
		After 1st week of illness: 10 mL urine (neutralized)	Sterile container, RT, immediately
	Leptospira antibody, microscopic agglutination test	Serum	Clot tube, RT, 2 h

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Fungal			
Cryptococcus neoformans, Cryptococcus gattii	*Cryptococcus* antigen test	Cerebrospinal fluid	Sterile container, RT, 2 h
	Gram stain Aerobic bacterial culture (faster growth on blood agar medium) Fungal culture	Cerebrospinal fluid	Sterile container, RT, 2 h
Coccidioides spp	*Coccidioides* antibody, complement fixation and immunodiffusion[5]	Cerebrospinal fluid	Sterile container, RT, 2 h
		Serum	Clot tube, RT, 2 h
	Coccidioides antigen test	Cerebrospinal fluid	Sterile container, RT, 2 h
	Calcofluor stain and Fungal culture	Cerebrospinal fluid	Sterile container, RT, 2 h
Parasitic			
Naegleria fowleri	See Table 7- Encephalitis		
Viral			
Enteroviruses (nonpolio)	Enterovirus NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Parechoviruses	Parechovirus NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Herpes simplex virus (HSV)	HSV 1 and 2 NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Varicella-zoster virus (VZV)	VZV NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Lymphocytic choriomeningitis virus (LCM)	LCM antibodies, IgM and IgG, IFA	Cerebrospinal fluid	Closed container, RT, 2 h
		Serum	Clot tube, RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
		Cerebrospinal fluid	Closed container, RT, 2 h
	Mumps culture and NAAT[4]	Cerebrospinal fluid	Sterile container, on ice, immediately
		Buccal or oral swab[6]	Viral transport device,on ice, immediately
Human immunodeficiency virus (HIV)	See footnote 7.		

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

[2]A negative result does not rule out *M. tuberculosis*.

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

[4]CDC accepts specimens referred by state or local public health laboratories: https://www.cdc.gov/laboratory/specimen-submission/index.html

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

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

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[7]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 (a mild CSF lymphocytosis with a mildly elevated CSF protein level and normal glucose) combined with definitive evidence of recent HIV infection (see Section XVI – VIRAL SYNDROMES; HIV diagnosis).

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 1998-2005 (69% viral, 20% bacterial, 7% prion, 3% parasitic, 1% fungal); a possible cause was identified for an additional 13% of patients [40]. 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 [41].

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 on CSF for HSV encephalitis are >95%; early data showed that HSV is cultured from CSF in <5% of cases [42, 43]. Reports of false negative HSV NAAT are the basis of recommendations to collect another CSF specimen 3-7 days later for repeat testing if HSV encephalitis continues to be suspected [41, 44]. 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) [43]. Additional NAAT specific for Parechoviruses is recommended for young children [44]. Because the performance characteristics of molecular testing for other causes of viral encephalitis are not well established, serology and repeat molecular testing may be required (Table 7).

Table 7. Laboratory Diagnosis of Encephalitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Viral			
Herpes simplex virus (HSV)	HSV 1 and 2 NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Enteroviruses (nonpolio)	Enterovirus NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Parechoviruses	Parechovirus NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
West Nile virus (WNV)	WNV IgM antibody[1]	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	WNV NAAT (low sensitivity)[2]	Cerebrospinal fluid and/or serum	Sterile container, RT, 2 h

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Other arboviruses[3]	Virus specific antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Varicella-zoster virus (VZV)[4]	VZV NAAT	Cerebrospinal fluid or plasma	Sterile container or EDTA tube, RT, 2 h
	VZV antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Epstein-Barr virus (EBV)	EBV NAAT[5]	Cerebrospinal fluid or plasma	Sterile container or EDTA tube, RT, 2 h
	EBV antibodies, VCA IgG and IgM, EBNA	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Cytomegalovirus (CMV)[6]	CMV NAAT[7]	Cerebrospinal fluid or plasma	Sterile container or EDTA tube, RT, 2 h
	CMV antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Human herpes virus 6 (HHV-6)	HHV-6 NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
JC virus	JC virus NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Mumps virus	Mumps virus antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
		Cerebrospinal fluid	Closed container, RT, 2 h
	Mumps culture and NAAT	Cerebrospinal fluid	Sterile container, on ice, immediately
		Buccal or oral swab	Viral transport device, on ice, immediately
Measles (Rubeola) virus	Measles antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	Measles culture and NAAT	Cerebrospinal fluid, urine	Sterile container, RT, 2 h
		Nasopharyngeal or 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 plasma	Sterile container or EDTA, RT, 2 h

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Rabies virus[8]	Rabies antigen, DFA	Nuchal skin biopsy	Closed container, RT, immediately
	Rabies NAAT	Saliva	Sterile container, RT, immediately
	Rabies antibody	Cerebrospinal fluid and serum	Closed container, clot tube, RT, 2 h
Lymphocytic choriomeningitis virus (LCM)	LCM antibodies, IgM and IgG, IFA	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Zika (see Table 73)			
Bacterial			
Mycobacterium tuberculosis	See Table 6- Meningitis		
Bartonella spp	*Bartonella* spp NAAT	Cerebrospinal fluid or plasma	Sterile container or EDTA, RT, 2 h
	Bartonella spp antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Mycoplasma pneumoniae	*M. pneumoniae* NAAT	Cerebrospinal fluid or respiratory	Sterile container, RT, 2 h
	M. pneumoniae antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Tropheryma whipplei (Whipple's Disease)	*Tropheryma whipplei* NAAT	Cerebrospinal fluid	Sterile container, RT, 2 h
Listeria monocytogenes	Gram stain Aerobic bacterial culture	Cerebrospinal fluid, blood	Sterile container, aerobic blood culture bottle, RT, 2 h
	Listeria antibody, CF	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
Coxiella burnetii (Q fever)	*C. burnetii* antibodies, IgM and IgG	Serum	Clot tube, RT, 2 h
	C. burnetii NAAT	Whole blood	EDTA tube, RT, 2 h
		Tissue	Sterile container, RT, 2 h
Rickettsia rickettsii (Rocky Mountain spotted fever, RMSF), *R. typhi*	*Rickettsia* spp antibodies, IgG and IgM, IFA	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	R. rickettsii DFA or IHC and NAAT	Skin biopsy from rash	Sterile container, RT, 2 h
	R. rickettsii NAAT	Whole blood	EDTA tube, RT, 2 h

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Ehrlichia chaffeensis, Anaplasma phagocytophilum	*E. chaffeensis* and *A. phagocytophilum* antibodies, IgM and IgG	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	E. chaffeensis and *A. phagocytophilum* NAAT	Whole blood	EDTA tube, RT, 2 h
Other: *B. burgdorferi, T. pallidum, Leptospira* spp	See Table 6 – Meningitis		
Fungal			
Cryptococcus neoformans, Cryptococcus gattii	Cryptococcus antigen test	Cerebrospinal fluid, serum	Closed container,clot tube, RT, 2 h
	Gram stain Aerobic bacterial culture Fungal culture	Cerebrospinal fluid	Sterile container, RT, 2 h
Coccidioides species	*Coccidioides* antibody, immunodiffusion and complement fixation[9]	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	Calcofluor stain Fungal culture	Cerebrospinal fluid, other sites	Sterile container, RT, 2 h
	Histologic examination	Tissue or formalin-fixed tissue	Sterile container, RT, 2 h or formalin, indefinite
Parasitic			
Acanthamoeba spp *Naegleria fowleri*	Microscopic wet mount Giemsa stain	Cerebrospinal fluid	Closed container, RT, 2 h
	Histology (trichrome stain)	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h
	Culture	Cerebrospinal fluid, brain tissue	Sterile container, RT, 2 h
	Acanthamoeba antibody IFA[10]	Serum	Clot tube, RT, 2 h
	Acanthamoeba IIF staining[10]	Brain tissue	Closed container, RT, 2 h
	NAAT[10]	Cerebrospinal fluid, brain tissue	Sterile container, RT, 2 h
Balamuthia mandrillaris	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h
	Balamuthia antibody, IFA[10]	Serum	Clot tube, RT, 2 h
	Balamuthia IIF staining[10]	Brain tissue	Closed container, RT, 2 h
	NAAT[10]	Cerebrospinal fluid, brain tissue	Sterile container, RT, 2 h
Baylisascaris procyonis[11]	*B. procyonis* antibodies	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h

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Trypanosoma brucei	Giemsa stain	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h
		Blood	EDTA tube, RT, 2 h
Toxoplasma gondii	*Toxoplasma* NAAT	Cerebrospinal fluid, serum, plasma	Sterile container, clot tube, EDTA tube, RT, 2 h
	Toxoplasma antibodies, IgM and IgG[12]	Cerebrospinal fluid and/or serum	Closed container or clot tube, RT, 2 h
	Giemsa stain, histology	Cerebrospinal fluid, brain tissue	Closed container, RT, 2 h
Prion			
Creutzfeldt-Jakob disease[13]	Real-time quaking-induced conversion (RT-QuIC)	Cerebrospinal fluid	Closed container, RT, 2 h
	Neuron-specific enolase (NSE)	Cerebrospinal fluid	Closed container, RT, 2 h
	Tau	Cerebrospinal fluid	Closed container, RT, 2h
	14-3-3 protein	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[14]
	Western blot for prion protein	Frozen brain tissue	Contact surgical pathologist prior to collection of tissue[14]
	PRNP gene sequencing	Blood, other tissues	EDTA tube, sterile container, RT, 2 h

[1]DFA, direct fluorescent antibody; IFA, indirect fluorescent antibody; RT, room temperature; IIF, indirect immunofluorescent antibody.

[2]WNV IgM antibody may persist for >6 months. False positives may occur with recent immunization (Japanese encephalitis, yellow fever) or other flavivirus infection (dengue, St. Louis encephalitis, Zika); confirm detection of IgM in CSF with plaque-reduction neutralization testing or NAAT [45].

[3]Sensitivity of WNV NAAT in immunocompetent host is <60% [45]. Testing for IgM in CSF is preferred, but may be falsely negative during first week of symptoms.

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

[5]Detection of VZV DNA in CSF (~60% of cases), CSF IgM, or intrathecal antibody synthesis distinguishes meningoencephalitis from post infectious, immune-mediated process [43].

[6]Quantitative EBV NAAT may help distinguish true positive from latent virus [43].

[7]Congenital disease in newborns and reactivation in immunocompromised hosts. False positive CSF CMV NAAT results have been reported in immunocompetent patients with bacterial meningitis [43].

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[8]In HIV patients, detection of CMV DNA in CSF has 82-100% sensitivity and 86-100% specificity for diagnosing CNS CMV infection [43].
[9]Contact state or local public health department to arrange testing; Questions regarding sampling techniques and shipping may be directed to the Rabies Duty Officer at the Centers for Disease Control and Prevention (404)-639-1050.
[10]Complement fixation on CSF is optimal test; serum complement fixation antibody may reflect a remote rather than an active infection.
[11]Available at the Centers for Disease Control and Prevention; for pre-mortem diagnosis contact CDC Emergency Operations Center (770) 488-7100 [46]. https://www.cdc.gov/laboratory/specimen-submission/index.html
[12]Consider if eosinophilia or exposure to raccoon feces [47]. Testing available at CDC https://www.cdc.gov/laboratory/specimen-submission/index.html
[13]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) [48].
[14]Testing available at the National Prion Disease Pathology Surveillance Center (NPDPSC) http://www.cjdsurveillance.com. RT-QuIC is >90% sensitive, >98% specific for prion disease [45, 46]. The 14-3-3, NSE, and tau protein are nonspecific markers for prion disease.
[15]Compliance with appropriate infection control protocols is essential https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf.

Focal Infections of Brain Parenchyma

Focal parenchymal brain infections start as cerebritis, then progress to necrosis surrounded by a fibrous capsule. There are two 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 8). A wider array of organisms is encountered in immunocompromised individuals.

Table 8. Laboratory Diagnosis of Focal Parenchymal Brain Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			
Aerobes: *Streptococcus* (especially *S. intermedius*), *Staphylococcus*, Enterobacterales, *Pseudomonas*, *Haemophilus*, *Listeria* spp Anaerobes: *Bacteroides*, *Fusobacterium*, *Prevotella*, *Actinomyces*,	Gram stain Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/sequencing	Aspirate of abscess contents, tissue	Sterile anaerobic container, RT, immediately

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Clostridium, Cutibacterium spp			
Nocardia spp	Gram stain, modified acid fast stain Aerobic bacterial culture (hold 14 d; add buffered charcoal yeast extract (BCYE) agar) 16S ribosomal RNA gene PCR/sequencing	Aspirate of abscess contents, tissue	Sterile container, RT, immediately
	Histology (Gomori Methenamine Silver (GMS), Gram stain)	Tissue	Closed container, RT, 2 h
Mycobacterium tuberculosis	AFB smear AFB culture	Aspirate of abscess contents (no swabs), tissue	Sterile container, RT, 2 h
	Histology (AFB stain)	Tissue	Closed container, RT, 2 h
	M. tuberculosis NAAT[1] 16S ribosomal RNA gene PCR/sequencing	Aspirate, tissue	Sterile container, RT, 2 h
Fungal			
Candida spp *Cryptococcus* spp *Aspergillus* spp *Mucorales (Rhizopus, Mucor* sp*)* *Pseudoallescheria boydii (Scedosporium apiospermum)* *Trichosporon* spp *Trichoderma* spp Dematiaceous moulds *(Cladiophialophora bantiana, Bipolaris* spp, *Exophiala* spp Endemic dimorphic fungi	Calcofluor stain Fungal culture	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Histology (GMS stain) Mucicarmine stain for *Cryptococcus*	Tissue	Closed container, RT, 2 h
Parasitic			
Toxoplasma gondii	*Toxoplasma* NAAT	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Toxoplasma antibodies, IgM and IgG[2]	Serum	Clot tube, RT, 2 h
	Giemsa stain Histology	Aspirate of abscess contents, tissue	Closed container, RT, 2 h Formalin, indefinite

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Taenia solium (neurocysticercosis)	*T. solium* antibodies, IgG, ELISA, confirmatory Western blot[3]	Serum	Clot tube, RT, 2 h
	Histology[4]	Brain tissue	Closed container, RT, 2 h Formalin, indefinite
Acanthamoeba spp *Naegleria fowleri*	Microscopic wet mount Giemsa stain	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	Histology (trichrome stain)	Aspirate of abscess contents, tissue	Closed container, RT, 2 h
	Culture	Aspirate of abscess contents, tissue	Sterile container, RT, 2 h
	Acanthamoeba antibody, IFA[5]	Serum	Clot tube, RT, 2 h
	Acanthamoeba IIF staining[5]	Brain tissue	Closed container, RT, 2 h
	NAAT[5]	Cerebrospinal fluid, Brain tissue	Closed container, RT, 2 h
Balamuthia mandrillaris	Histology (trichrome stain)	Brain tissue	Closed container, RT, 2 h Formalin, indefinite
	Balamuthia antibody, IFA[5]	Serum	Clot tube, RT, 2 h
	Balamuthia IIF staining[5]	Brain tissue	Closed container, RT, 2 h
	NAAT[5]	Brain tissue	Closed container, RT, 2 h

[1]A negative result does not rule out *M. tuberculosis*.

[2.]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 [48].

[3]Only 50% sensitivity if patient has solitary parenchymal lesion [49]; potential for false positive ELISA results due to cross reactivity with Echinococcus.

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

[5]Available at the Centers for Disease Control and Prevention; for pre-mortem diagnosis contact CDC Emergency Operations Center (770) 488-7100 [50]. https://www.cdc.gov/laboratory/specimen-submission/index.html

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. Four to 17% of shunts become infected (Table 9) [50]. Potential routes of shunt infection include contamination at time of placement, contamination from the distal portion (retrograde), breakdown of the skin over the shunt, and hematogenous seeding. Blood cultures should also be collected if the shunt terminates in a vascular space (ventriculoatrial shunt). Most CNS shunt infections are caused by bacteria. Fungi are more likely to cause shunt infections in immunocompromised patients and those receiving total parenteral nutrition, steroids, or broad-spectrum antibiotics. Culture of shunt or drain components after removal should not be performed unless the patient has symptoms of a CNS infection [50].

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Table 9. Laboratory Diagnosis of Central Nervous System Shunt Infections

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

Subdural Empyema, Epidural Abscess, and Suppurative Intracranial Thrombophlebitis

Cranial subdural empyema and cranial epidural abscess are neurosurgical emergencies that are usually caused by bacteria (streptococci, staphylococci, aerobic gram-negative bacilli, anaerobes, often polymicrobial) (Table 10). Mycobacteria and fungi are rare 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.

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

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			

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Aerobes: *Streptococcus, Enterococcus, Staphylococcus,* Enterobacterales, *Haemophilus, Pseudomonas* spp Anaerobes: *Peptostreptococcus, Veillonella, Bacteroides, Fusobacterium, Prevotella* spp, *Cutibacterium acnes*	Gram stain Aerobic and anaerobic bacterial culture	Aspirate of purulent material (no swabs)	Sterile, anaerobic container, RT, immediately
Nocardia spp	Gram stain, modified acid fast stain Aerobic bacterial culture (hold 7 d; add BCYE agar) 16S ribosomal RNA gene PCR/sequencing	Aspirate of purulent material (no swabs)	Sterile container, RT, immediately
Mycobacterium spp	AFB smear AFB culture *M. tuberculosis* NAAT[1] (rarely available) 16S ribosomal RNA gene PCR/sequencing	Aspirate of purulent material (no swabs)	Sterile container, RT, 2 h
Fungal			
Candida spp, other fungi	Calcofluor stain Fungal culture	Aspirate of purulent material (no swabs)	Sterile container, RT, 2 h

[1]Negative NAAT for tuberculosis does not rule out *M. tuberculosis*.

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 [51]. The differential diagnosis of soft-tissue infections of the head and neck frequently includes pharyngitis, otitis media, and sinusitis; those are covered in section VII. Odontogenic infections are caused commonly by periodontal or gingival anaerobic microbiota [52]. These infections include peritonsillar and pharyngeal abscesses, deep space abscesses, such as those of the retropharyngeal, parapharyngeal, submandibular and sublingual spaces, dental abscesses and cervical lymphadenitis [53, 54]. Complications of odontogenic infection can occur by hematogenous spread or by direct extension resulting in septic jugular vein thrombophlebitis (Lemierre's syndrome), bacterial endocarditis, intracranial abscess, or acute mediastinitis [55, 56]. Accurate etiologic diagnosis depends upon needle aspiration or open drainage of inflammatory material from infected tissues and tissue spaces while avoiding contamination with mucosal microbiota [57, 58]. The specimen should be placed

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into an anaerobic transport container to support the recovery of both anaerobic and aerobic bacteria [57]. Requests for Gram stained smears should accompany all culture requests because Gram stain results allow the laboratorian to evaluate the adequacy of the specimen by identifying inflammatory cells, provide an early, presumptive etiologic diagnosis, and identify morphologic patterns indicative of mixed aerobic and anaerobic infection [59]. Additionally, spirochetes, and some anaerobic etiologies involved in odontogenic infections such as necrotizing gingivitis (a.k.a. trench mouth and Vincent's angina), cannot be recovered in routine anaerobic cultures but will be seen in the Gram stained smear [60]. The use of targeted NAAT testing results in the identification of a greater number of anaerobic species than detected by culture in most specimens, but is not appropriate as a routine because the additional information does not impact diagnosis or selection of antimicrobial therapies [61].

Infections caused by oropharyngeal microbiota include epiglottitis, mastoiditis, inflammation of salivary tissue and suppurative parotitis [51, 62]. 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, including aerobic and anaerobic media, are the preferred sample for the diagnosis of epiglottitis, being positive in 70% of 40 confirmed cases that included Hib vaccinated and unvaccinated children, and positive in 27% of 79 Hib unvaccinated adults with epiglottis [63, 64]. If swabbing of the epiglottis is attempted, it should be in a setting with available appropriate emergency response [65]. Oropharyngeal microbiota also can extend into tissues of the middle ear and mastoid [51, 66]. Aspirated material, saline lavage of a closed space, and tissue or tissue scrapings are preferred specimens, and must be transported in a sterile container. Small pieces of tissue must be kept moist during transport. This can be accomplished by adding a few drops of sterile, non-bacteriostatic, normal saline. Suppurative parotitis is sampled by expressing pus from the parotid gland followed by collection by aspiration. Swabbing is susceptible to contamination by adjacent bacterial microbiota, although, swabbing for mumps virus NAAT testing is the preferred sample. In the rare instance where anaerobic bacterial pathogens are suspected (especially chronic middle ear, mastoid or parotid infection), anaerobic transport is required.

Infections caused by exogenous pathogens (not part of the oral microbiota) include malignant otitis externa, otitis externa, animal bites, trauma, irradiation burns and complications of surgical procedures [66, 67]. Squamous epithelial microbiota and environmental pathogens are important etiologies of these infections; most frequently Gram-negative bacilli, such as *Pseudomonas aeruginosa,* and staphylococci. Aerobic and anaerobic bacterial culture, and occasionally fungal and mycobacterial culture, are needed to determine the specific etiology. Gram, fungal and mycobacterial stains should be ordered with corresponding cultures.

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Key points for the laboratory diagnosis of head and neck soft tissue infections:
o A swab is not the specimen of choice for these infections because of small volumes collected and ease of contamination with heavily colonized surfaces. Submit tissue, fluid, or aspirate when possible. This may require surgery with or without imaging guidance o Resist swabbing in cases of epiglottitis o Use anaerobic transport containers if anaerobes are suspected. This includes most specimens o Keep tissue specimens moist during transport

Tables 11 and 12 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.

Table 11. Laboratory Diagnosis of Infections of the Oral Cavity, and Adjacent Spaces and Tissues Caused by Gingival, Periodontal and Oropharyngeal Microbiota

Disease and Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Necrotizing Gingivitis (Trench Mouth/Vincent's Angina)			
Mixed infection due to *Fusobacterium* spp. anaerobes and commensal spirochetes representing periodontal and gingival microbiota	Gram stain; culture not recommended	Biopsy or irrigation and aspiration of lesion; swab not recommended	Gram stain requires sterile container, RT, 2 h. If culture attempted, anaerobic transport vial, RT, 2 h
Dental Abscesses			
Periapical, periodontal and gingival abscesses caused by periodontal/gingival microbiota diagnosed and treated by dentists	Gram stain and culture not routinely performed, when necessary aerobic and anaerobic bacterial culture	If performed, biopsy, aspiration ,or irrigation and aspiration of abscess	If culture attempted, anaerobic transport vial, RT, 2 h
Epiglottitis and Supraglottitis			
Normal Host			
Haemophilus influenzae *Streptococcus pneumoniae* Beta-hemolytic streptococci *Staphylococcus aureus* *Neisseria meningitidis*	Gram stain Aerobic bacterial culture	Clinical diagnosis may not require specimen to confirm Swab of epiglottis[1] only if necessary	Swab transport device, RT, 2 h
	Blood cultures	Blood, 2-4 sets	Aerobic and anaerobic blood

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Caused by bacterial microbiota of the oropharynx			culture bottles, RT, immediately
Immunocompromised Host			
Same bacteria as in the normal host above but also other agents such as *Pasteurella multocida*	Gram stain Aerobic bacterial culture	Clinical diagnosis may not require specimen to confirm Swab of epiglottis[1] only if necessary	Swab transport device, RT, 2 h
	Blood cultures	Blood, 2-4 sets	Aerobic and anaerobic blood culture bottles, RT, immediately
Aspergillus spp Other filamentous fungi	Calcofluor-KOH stain Fungal culture	Biopsy or specimen collection during fiberoptic laryngoscopy Swab much less likely to recover fungi	Sterile container, RT, 2 h
	Fungal blood cultures	Blood, 2-4 sets	Aerobic blood culture bottle formulated for fungi, RT, immediately
Peritonsillar/Pharyngeal Abscess			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus* group *Arcanobacterium haemolyticum* Mixed aerobic and anaerobic bacterial microbiota of the gingiva and oral cavity	Gram stain Aerobic and anaerobic bacterial culture (for pharyngitis and throat swabs, refer to Section VII)	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile anaerobic container, RT, 2 h
Lemierre Syndrome			
Fusobacterium necrophorum Occasionally mixed anaerobic bacterial microbiota of the oral cavity including *Prevotella* spp and anaerobic Gram-positive cocci Caused by tonsillar, gingival and	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of lesion; swab not recommended	Sterile anaerobic container, RT, 2 h
	Blood cultures[2]	Blood, 2-4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately

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oropharyngeal microbiota			
Mastoiditis			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Streptococcus pyogenes* *Staphylococcus aureus* *Pseudomonas aeruginosa* Enterobacterales Anaerobic bacteria Caused by oropharyngeal microbiota	Gram stain Aerobic and anaerobic bacterial culture	Middle ear fluid obtained by tympanocentesis or biopsy of mastoid tissue; swab not recommended (for a discussion of acute otitis media refer to Section VII)	Sterile anaerobic container, RT, 2 h
Mycobacterium tuberculosis	Acid fast stain AFB culture Targeted NAAT	Biopsy of mastoid tissue	Sterile container, RT, 2 h
Submandibular, Retropharyngeal and Other Deep Space Infections Including Ludwig's Angina			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus* group *Actinomyces* spp Mixed aerobic and anaerobic bacteria All originating from gingival and oropharyngeal microbiota	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of lesion; swab not recommended	Sterile anaerobic container, RT, 2 h
	Blood cultures[2]	Blood, 2-4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately
Cervical Lymphadenitis – Acute Infection			
Streptococcus pyogenes *Staphylococcus aureus* *Streptococcus anginosus* group Mixed aerobic and anaerobic bacteria All originating from gingival and oropharyngeal microbiota	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile anaerobic container, RT, 2 h
	Blood cultures[2]	Blood, 2-4 sets	Aerobic and anaerobic blood culture bottle, RT, immediately
Cervical Lymphadenitis - Chronic Infection			
Mycobacterium avium complex *M. tuberculosis*	Acid fast stain AFB culture NAAT for mycobacteria	Biopsy, aspiration or irrigation of abscess;	Sterile container, RT, 2 h

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Other mycobacteria Originating from oropharyngeal sources		swab not recommended	
Listeria monocytogenes Originating from oropharyngeal sources	Gram stain Aerobic and anaerobic bacterial culture	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, 2 h
Bartonella henselae Originating from cutaneous sources outside of the oropharynx	*Bartonella* NAAT[3]	Tissue or aspirate 5 mL plasma	Sterile container, RT, 2 h EDTA tube, RT, 2 h
	Bartonella culture not recommended as a rourtine[4] Histopathology (Warthin-Starry and H&E stains) *Bartonella* serology	If performed, biopsy, aspiration or irrigation of abscess; swab not recommended Tissue in formalin for histopathology Clotted blood/serum for serology	Sterile container, RT, 2 h Container for pathology, indefinite Sterile tube containing clotted blood. Remove sera and refrigerate

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

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

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

[4]Bartonella culture is not recommended. If requested, the laboratory should be alerted 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. Bartonella serology is often helpful.

Table 12. Laboratory Diagnosis of Malignant Otitis Externa and other Infections Caused by Exogenous Pathogens

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Malignant Otitis Externa			
Pseudomonas aeruginosa	Gram stain Aerobic bacterial culture	Scraping or fluid from external canal or tissue biopsy from temporal bone or mastoid	Sterile container, RT, 2 h
Otitis Externa			
Filamentous Fungus such as *Aspergillus niger*	Fungal stain Fungal culture	Scraping or fluid from external canal	Sterile container, RT, 2 h
Animal Bites, Trauma, Irradiation Burns and Surgical Complications			

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Microbiota sources including squamous epithelium of the head, animal mouth and the environment	Gram stain plus aerobic and anaerobic culture Fungal stain and culture, and mycobacterial stain and culture when appropriate, e.g., soil/plant material introduced by trauma	Biopsy, aspiration or irrigation of abscess; swab not recommended	Sterile container, RT, 2 h

V. Ocular infections

The variety and complexity of ocular infections continues to increase with the advancement of ophthalmologic interventions, emerging ocular pathogens, increasing antimicrobial resistance, and novel antimicrobial agents. The spectrum of disease ranges from superficial infections treated topically to sight-threatening deep-seated infections requiring aggressive surgical intervention and parenteral antimicrobials. The microanatomy of the eye is complex with subtleties in the etiologic agents causing infection at each site and diagnostic recommendations are often based on studies with a small number of clinical specimens [68]. This includes infections of anatomical structures surrounding the eye (conjunctivitis, blepharitis, canaliculitis, dacryocystitis, orbital and periorbital cellulitis), on the ocular surface (keratitis), and within ocular fluids and internal structures (endophthalmitis and uveitis/retinitis) [68]. Some diagnoses can be made clinically on the basis of ocular examination; however, the clinical microbiology lab plays an ever-increasing role in optimizing the diagnostic yield from small volume and often irreplaceable ocular samples [68].

Challenges associated with the diagnosis of ocular infections include: limited sample volume, irreplaceable specimens, bedside inoculation, antimicrobial exposure prior to sample collection, diverse pathogens requiring specific collection and transport conditions, logistics solutions required for prompt transport from remote sites, and limited ocular anatomy/pathophysiology training. Close interaction between ophthalmologists and microbiologists is critical to address these challenges.

Key points for the lab diagnosis of ocular infections:

- Label specimens with the specific anatomic source, e.g. right conjunctiva; not just "eye".
- Given small volume, communication is critical to prioritize and optimize diagnostic yield.
- Consult the lab regarding suspicious agents (e.g. *Neisseria gonorrhoeae*).
- If bedside inoculation is performed, proper aseptic technique and the use of unexpired media and collection devices is essential for optimal organism recovery.
- Despite their occasional use, swabs are suboptimal for ocular surfaces; corneal scrapings and ocular fluids are preferred.

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- A paired ocular surface specimen from the uninfected eye can be used as a "control" to assist in culture or Gram stain interpretation of commensal microbiota.
- There are no FDA-approved molecular diagnostic tests for ocular specimens but specialized labs offer lab-developed NAATs and 16S ribosomal gene PCR/sequencing.
- Helpful interventions include: annual ocular diagnostics education, a designated clinic contact and system to restock media, specialized courier services, and availability of microbiology consultative services.

Specimen Collection and Transport

The *source*, *type*, and *suspected infection* is essential to triage small volume specimens to achieve clinically actionable results. If this information is not received, providers must be contacted for additional clarification. This is particularly important because all major pathogen groups --viruses, parasites, bacteria, mycobacteria, and fungi can cause ocular infection [68]. Both epidemiology and clinical presentation are used to narrow the organism(s) sought and the lab tests requested [68]. If less than adequate volume is received, a report comment can be added to state "Specimen cultured; volume inadequate for optimal recovery". Labs should make every effort to contact providers before or upon cancellation of orders. Ophthalmologists collect most specimens which include: swabs of ulcers, corneal scrapings, biopsies, anterior chamber aspirates, or vitreous aspirates /washings [68].

Given limited ocular fluid volume and an increasingly complex test menu, sample submission directly to the lab is preferred [68]. Given the small volume, the lab and ophthalmology may agree to inoculate specimens and smears at the bedside. In this case, the lab should establish a system to supply media and slides for ready access by the surgeon and work together with providers to ensure materials do not out-date and meet all quality control standards [68]. Development of diagnostic kits with clearly-labeled media and collection devices, as well as instructions on how to prepare slides and inoculate plates can optimize specimen collection. The choice of media and order of inoculation of plates should be determined in consultation with the lab and follow updated practical guidance recently reviewed by Leal *et. al* [68]. Also, provide the order of inoculation of media for the clinicians in the kit instructions. To avoid confusion in ordering, reporting, and result interpretation, separate sources should have separate plates and be analyzed separately by the lab. Despite their occasional use, inoculation of the same plate with multiple sources using distinct patterns to indicate the source is suboptimal (e.g. horizontal and vertical streaks for right and left conjunctiva, respectively; "R", "L", and "C" patterns for right conjunctiva, left conjunctiva, and cornea, respectively) [68].

Anterior chamber or vitreous fluid aspirates are optimal for the detection of intraocular infection. Fluids should be transported in their original containers (e.g., syringe with the needle

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removed) or expressed into a sterile specimen container of appropriate size to avoid unnecessary specimen loss (e.g., side of container, oversized syringes) [68]. Tissues, foreign objects, artificial lens, and specimens collected on sterile filter paper should be transported intact and moistened with sterile saline [68]. If anaerobic culture is desired, fluids should be transported in a closed syringe without excess gas and delivered to the lab as soon as possible [68]. Tissues should be placed in an anaerobic transport device if transit will take >2 h. Viral culture is indicated only for refractory cases and samples should be submitted in universal transport media and transported at room temperature within 2h [68].

Direct microscopic examination including Gram stain, calcofluor white for fungi and *Acanthamoeba,* or direct fluorescent antibody (DFA) for *Chlamydia trachomatis*, may be useful in preliminary diagnoses. Given small volumes, tissues must be placed within a 1-cm^2 demarcated area on a clean frosted glass slide and transported to the lab in a plastic slide holder [68]. Dust and cardboard material can introduce artifacts and fibers that autofluoresce or bind reagents, like calcofluor white, complicating accurate slide interpretation [68]. One slide is required for each requested stain. Cytocentrifugation is used to concentrate ocular fluids prior to direct exam.

Although no molecular test is FDA approved for use on ocular specimens, nucleic acid amplification tests (NAAT) are recommended for the diagnosis of ocular *Chlamydia* and viral infections and are available at many academic medical centers serving large ophthalmology practices and commercial reference labs [68]. Ocular dyes and anesthetics inhibit both NAATs and viral culture and it is therefore critical to rinse the eye thoroughly with sterile, non-bacteriostatic saline prior to sampling [68]. Although eSwab solution promotes the survival of anaerobic and fastidious bacteria, it also exhibits host cell cytotoxicity and should not be used for viral culture [63, 64]. Similarly, oils in cotton swabs interfere with NAAT testing [68]. Universal Transport Media (UTM) is recommended for viral culture. If swabs are used, only dacron, rayon, and flocculated swabs are acceptable for molecular testing. Sample collection and transport recommendations for organism sequencing are the same as described for other molecular tests [68].

Sample processing

It is helpful to think of the lab diagnosis of ocular infections using a dichotomous classification system which clearly identifies ocular sites in which the isolation of commensal microbiota may represent normal microbiota (Group 1- Outer Eye) from tissues in which isolation of these organisms is more likely to represent true infection (Group 2- Inner Eye).

Group 1-Outer Eye infections involve non-sterile outer eye structures: conjunctivitis, dacryocystitis, blepharitis, canaliculitis, and pre-septal and septal cellulitis. Culture of purulent lesions and expressed concretions from within clogged ducts are highest yield, particularly for anaerobic bacteria, such as *Actinomyces* [68]. Cotton and calcium alginate swabs should not be used as fatty acids in the cotton fibers and glue in calcium alginate swabs inhibit bacterial growth,

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particularly *Neisseria* species [68]. Limited data exists on the use of flocculated swabs but as for other sample sites, increased adsorption is expected with this technology [69]. Dry lesion sampling may benefit from the use of swabs pre-moistened with sterile saline [68]. Table 13 highlights the most common pathogens in Group 1-Outer Eye specimens.

Group 2- Inner Eye infections involve sterile inner eye structures: endophthalmitis, keratitis, anterior uveitis (anterior chamber), posterior uveitis (posterior chamber), and retinitis. Although the cornea is part of the ocular surface, it is treated as a sterile site and categorized as Group 2 [68]. Specimens are obtained via invasive methods and include tissues, biopsies, scrapings, aspirates, ocular fluids, and surgical irrigation fluid. Scalpel blades, Kimura platinum spatulas, and 25, 27 or 30-gauge needles (sometimes bent at the tip) are often utilized and can be used to inoculate liquid or solid media directly [68]. Table 14 and 15 highlight the most common pathogens in Group 2-Inner Eye specimens.

Direct Exam- Gram stains have a high positive predictive value but low sensitivity due to limited sample volume and prior antimicrobial treatment [68]. Labs should note the potential for artifacts such as small round pigmented iris melanin granules to mimic bacterial cocci [68]. Modified acid fast and acid-fast stains are rarely used but may be helpful to identify *Nocardia* and mycobacteria, particularly post-LASIK surgery or traumatic keratitis unresponsive to standard antimicrobials [68, 70]. Giemsa stains may show intracellular inclusion bodies associated with *Chlamydia* infection, *Acanthamoeba* cysts, or viral cytopathic effects [68, 71, 72]. Calcofluor white stains highlight microsporidia, yeasts, hyphae, and amebic cysts [68, 73, 74].

Antigen Tests CLIA-waived enzyme immunoassays for adenovirus are available and may be helpful in limiting inappropriate antibiotic use, boosting provider confidence in the use of topical steroids, prompting the pursuit of alternative diagnoses, and clearing patients to safely return to work [75-77].

Culture To simplify culture approaches, Group 1 and 2 specimens should be inoculated onto the same media and incubated the same length of time [68]. Corneal scrapings and ocular fluids are preferred over swabs [68]. Scrapings, swabs, and small volume fluids (1-200µl) should be streaked for isolation and tissues minced using sterile scissors or a scalpel prior to inoculation on appropriate media [68]. If enough fluid is received (e.g., a wash or irrigations fluid), centrifuge the fluid, and use the sediment to inoculate the media or filter through a 0.45- or 0.22-µm sterile membrane filter and collect and culture the filter membrane [68]. Culture of vitreous fluid (0.1-3 ml) in pediatric blood culture bottles may further increase sensitivity compared to traditional culture, but this off-label use requires validation and is not routinely available [78, 79]. Most bacteria and yeasts associated with ocular infections will grow within 48-72 hr. Notify the lab of cases of suspected iatrogenic infection so that extended incubation periods are implemented to support *C. acnes* and mycobacterial isolation [68, 80]. It is also critical to notify the lab if there is suspicion for *N. gonorrhoeae* given the potential need to include additional selective media. Culture remains the gold standard to diagnose ocular TB [68, 78]. In the research setting, NAATs

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on ocular fluid exhibit 56% sensitivity compared to culture [68]. Viral culture is indicated only in refractory herpes simplex virus (HSV), varicella zoster virus (VZV), or cytomegalovirus (CMV) cases and is limited to specialized reference labs [68, 81]. *Acanthamoeba* can be cultured on bacterial lawns on non-nutritive agar in reference labs, but NAATs are faster and more sensitive [82]. Definitive diagnosis of contact lens-related infectious keratitis requires microbe isolation from infected tissue. Culture of contact lens case and accessory items are not recommended given high rates of transient colonization and the potential to erroneously ascribe infection to an organism requiring a different therapy regimen [68].

Cornea Rims Explanted donor corneas are stored in special media containing antibacterial but not antifungal agents. Cornea rim cultures detect yeasts that may proceed to cause clinically relevant fungal keratitis in organ recipients in a subset of culture positive patients (3-14%) [83]. Since the overall rate of post-surgical corneal transplant infections is very low and the expense of culturing all donor rims high, there are no formal recommendations to guide the use of cornea rim cultures and this decision should be made at the institutional level with input from both ophthalmology and microbiology [83]. Since post-transplant fungal infections occur most commonly in patients undergoing endothelial keratoplasty, it may be prudent to limit cornea rim fungal cultures to donor corneas used in this type of surgery [83-85]. All specimens should be sent to the lab with large samples immersed in sterile saline and smaller samples placed between two sterile gauze pads wet with saline [68].

Susceptibility Testing Antimicrobial concentrations used to treat ocular infections may be orders of magnitude higher than systemic drug administration. All FDA, CLSI, and EUCAST breakpoints use clinical outcome data from patients receiving systemic antimicrobials rendering these breakpoints not applicable to ocular isolates [86, 87]. Labs choosing to perform antimicrobial susceptibility testing on ocular isolates may therefore report the MIC value and range without interpretation [68]. Many drugs used to treat ocular infection, particularly topical agents, are not included on commercial susceptibility testing platforms, not routinely tested in clinical labs, and lack interpretive breakpoints [86, 87]. No interpretation should be provided for bacteria, yeasts, and molds [68]. Antiviral susceptibility testing is limited to specialized reference labs and may be helpful in refractory HSV, VZV, and CMV ocular infections [68, 88].

Molecular Detection Intraocular fluids should be analyzed directly for the presence of infectious agents [68]. There are no FDA-approved NAATs for ocular specimens and most clinical laboratories lack access to enough positive clinical samples to validate in-house or commercial assays. However, commercial reference laboratories and a subset of hospital-based laboratories servicing large ophthalmology practices offer molecular testing on ocular specimens utilizing validated lab developed tests (LDT), some of which are FDA-approved for other specimen types [68]. NAATs exhibit increased sensitivity for most ocular pathogens relative to traditional culture [68, 89]. Specificity for pathogens that are not a part of the normal ocular surface microbiota (e.g. *C. trachomatis*) is > 99%, however, specificity drops considerably with molecular detection of commensal microbiota (e.g. *C. acnes*) which may or may not be an innocent bystander [68].

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NAATs are particularly helpful in deciphering the etiology of culture-negative intraocular infections with fastidious organisms [68]. Many non-FDA approved singleplex and multiplex PCR assays are described in the literature. In brief, HSV and VZV NAATs are the most commonly used and exhibit high sensitivity and specificity for active infection [68]. NAAT detection of ubiquitous commensal viruses, such as CMV, EBV, and HHV6 requires careful clinical correlation given their presence in latency and active disease [68]. Ocular infection by measles [90], Zika [91], Dengue [92], Chikungunya [92], and Ebola [93] will most often present in the context of a characteristic clinical syndrome. In this setting, viral detection and/or serologic confirmation in other specimen types (usually blood) is sufficient evidence for ocular involvement. Regional public health labs and/or the CDC offer PCR for all of these infectious agents. Any clinical suspicion for Ebola, Measles, and other highly communicable pathogens requires rapid communication to public health partners and appropriate infection prevention measures [94]. Trachoma, due to *C. trachomatis*, is predominantly diagnosed based on clinical presentation, but commercial NAATs are also available to aid in rendering this diagnosis [95].

The literature exhibits several examples of the target-agnostic potential of next generation sequencing (NGS), specifically metagenomics, to identify unsuspected pathogens including intraocular rubella infection in an individual with a >20 year history of chronic bilateral idiopathic uveitis [96]. However, high costs and low test volumes limit implementation of NGS in most clinical microbiology labs. Although specialized labs associated with academic institutions and commercial reference laboratories have validated NGS for clinical use on CSF, blood, and respiratory specimens, the availability of NGS tests on ocular specimens for patient care is currently limited to specialty laboratories.

Serology Detection Ocular syphilis is diagnosed via clinical findings, a positive CSF VDRL, and high serum rapid plasma reagin (RPR) with treponemal specific antibody confirmation [97]. Confirmation of retinitis or uveitis due to *Borrelia burgdorferi* is best accomplished with clinical symptoms and compatible serum-based serology [98]. Chronic *Toxoplasma gondii* ocular infection is diagnosed via intra-ocular serology coupled with clinical findings, however, when antibody levels are low and organism burden is high, such as early acute infection or immunocompromised states, NAATs exhibit higher sensitivity [99, 100]. For a thorough review of approaches to diagnose rare entities including ocular helminth and arthropod infections, readers are referred to a recent review by Leal *et.al.* [68].

Pre-septal and Orbital Cellulitis

Table 13 highlights the most common pathogens causing preseptal and orbital cellulitis.

Pre-septal Cellulitis Pre-septal cellulitis is characterized by inflammation of the eyelids, conjunctiva, and surrounding skin without involvement of deeper tissues and no pain with eye movements. Symptoms include eyelid erythema, warmth, tenderness and fluctuant lymphedema or swelling that can extend over the nasal bridge to the opposite eyelids usually accompanied by

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low-grade fever and elevated white blood cell count. Often there is a history of sinusitis, insect bite, dacryocystitis, local skin abrasion, laceration, dental abscess or puncture wound [68]. Culture of open wounds, weeping vesicles, purulent nasal drainage, and conjunctival discharge often yield *S. aureus,* β-hemolytic streptococci, *S. pneumoniae, H. influenzae,* and/or *P. aeruginosa* with rapid treatment often yielding a good outcome [68].

Orbital cellulitis All cases of orbital cellulitis should be considered potential sight-threatening medical emergencies that require prompt diagnostic work-up and treatment. Orbital cellulitis is characterized by inflammation of deep periocular tissues resulting in severe clinical features like conjunctival edema and injection, restricted ocular motility, and pain on attempted eye movement [68]. Additional symptoms include blurred vision, headache, double vision, eyelid edema, erythema, warmth, tenderness, proptosis, fever, purulent discharge, and hypesthesia. Prevailing etiologies include: direct extension from the paranasal sinuses, dental abscesses, or skin in addition to traumatic injury, post-surgical complications, or hematogenous seeding. Treatment requires hospitalization for IV antibiotics, imaging, and immediate ophthalmology and/or otolaryngology, neurosurgery, or infectious disease consultation [68]. If the orbit is taut and cramped, an optic neuropathy is present, or the intraocular pressure is dangerously increased, immediate surgical intervention is indicated [68]. Culture of open wounds, purulent drainage, and tissue obtained during surgery is optimal to diagnose orbital cellulitis. Bacterial causes include Staphylococcus species, Streptococcus species, H. influenzae, P. aeruginosa, Bacteroides species, and other environmental Gram-negative rods [101, 102]. Failure to respond to IV antimicrobials and the formation of a subperiosteal abscess may necessitate surgical intervention to clear bacterial infection [68]. Surgical intervention is also indicated for invasive mold infections such as sinonasal mucormycosis which must be ruled out in patients with poorly controlled diabetes [68]. All patients with orbital cellulitis must be monitored for complications, including cavernous sinus thrombosis, meningitis, and extension into the brain parenchyma [101].

Infection of the Eyelids and Lacrimal System

Table 13 highlights the most common pathogens causing lacrimal and eyelid infections. Blepharitis, canaliculitis, and dacryocystitis are all superficial infections that are generally self-limited [68]. Cultures from these sites are rarely submitted for diagnostic work-up and often yield a predominance of commensal microbiota making it difficult to attribute a clear pathogenic role [68]. Gram-positive skin microbiota including *S. aureus*, other *Staphylococcus* species, *S. pneumoniae*, and diphtheroids as well as anaerobes such as *C. acnes* and *Actinomyces* spp, gram-negatives including *H. influenzae* and *P. aeruginosa*, rapidly growing mycobacteria, and less commonly *Candida* spp are implicated . Expressed concretions and mucopurulent material increase diagnostic specificity and are recommended [68]. Punctal plugs used to treat dry eye disease are associated with *P. aeruginosa* and Rapidly Growing Mycobacteria [103]. Commensal mites and lice are associated with blepharitis and these periocular structures are a relatively common site for human myiasis [104, 105].

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Conjunctivitis Table 13 highlights the most common pathogens causing conjunctivitis. Conjunctivitis can occur as an isolated condition or secondary to other causes of ocular inflammation. It is characterized by a "red eye" (i.e., significant conjunctival hyperemia) over the bulbar conjunctiva and/or palpebral/tarsal conjunctiva often associated with irritation and discharge [106]. Diagnostic testing is rarely indicated given characteristic clinical presentations [68]. Viral conjunctivitis is most commonly bilateral with serous, watery discharge and known sick contacts. Allergic conjunctivitis is uniformly bilateral with watery discharge and grayish, scant, stringy mucus with an associated situational exposure history. In contrast, bacterial conjunctivitis is typically unilateral with more purulent discharge, matting and adherence of eyelids on waking.

Adenovirus is the most common cause of viral conjunctivitis (34-80%) [106, 107]. Treatment includes symptomatic relief and avoidance of personal contacts, washing hands, and sharing of personal items. Severe infections with membrane formation or cornea involvement may merit topical corticosteroids . Point of care antigen tests are available and may be useful in the decision to use anti-inflammatory agents. *Corticosteroids enhance viral replication, promote superinfection, delay viral clearance, and can facilitate higher numbers of community epidemics of viral conjunctivitis.* Enteroviruses and Coxsackie viruses cause similar clinical syndromes [108]. Less commonly, HSV and VZV reactivation can involve the conjunctiva with severe infection meriting the use of systemic antiviral agents [109].

When tests are requested, bacterial culture is often compromised by the prior use of empiric antibacterial therapy [68]. In adults, *S. aureus, S. pneumoniae* and *H. influenzae* are the most frequent causes of bacterial conjunctivitis, while *H. influenzae, S. pneumoniae,* and *Moraxella* spp are most common in children [68]. Commensal microbiota such as CNS, *Corynebacterium* spp, and viridans streptococci are generally considered non-pathogenic "normal microbiota" when recovered from the conjunctiva and only severe or recalcitrant infections are treated with topical antimicrobials [68]. *C. trachomatis* is associated with neonatal and sexually transmitted conjunctivitis in developed nations and is the leading cause of infectious blindness (trachoma) in developing nations [110]. Rare infections have also been reported with *C. pneumoniae* and *C. psittaci* [111]. *N. gonorrhoeae* conjunctivitis is a rapidly progressive medical emergency [68]. High risk individuals include neonates, infants, and sexually active adults [68]. To mitigate rapid ocular damage every effort should be made for Gram stains from ocular specimens to be reported in < 1 hour [68]. Worldwide rates of *N. gonorrhoeae* infection are increasing, and strains have emerged that are resistant to standard antimicrobial agents [112-114]. Neonatal conjunctivitis is also caused by HSV inoculated at the time of vaginal delivery and although rare, *P. aeruginosa* can cause life-threatening neonatal conjunctivitis in hospitalized infants [68, 110]. Microsporidia infection mimics viral keratoconjunctivitis in immunosuppressed hosts and is associated with HIV, hot springs, swimming pools, contact sports, and soil [68, 115].

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Keratitis

Table 14 highlights the most common pathogens causing infectious keratitis. All patients with suspected infectious keratitis should be referred to an ophthalmologist immediately for diagnosis and treatment of this sight-threatening medical emergency. Keratitis is characterized by inflammation of the cornea [68]. It is the fourth leading cause of blindness globally and is associated with improper contact lens use, trauma, dry-eye, chronic ocular surface disease, use of topical corticosteroids, lid abnormalities, corneal hypesthesia, and iatrogenic post-surgical infection [116, 117]. Symptoms include redness with mild to severe pain, photophobia, decreased vision, and purulent discharge [68]. If allowed to progress, severe scarring, thinning, perforation, or endophthalmitis may develop and progress to irreversible blindness and/or rupture of the globe resulting in evisceration or enucleation [68]. It is important to note that the use of dyes and topical anesthetics may inhibit NAAT reactions used to diagnose keratitis and the eye surface should be thoroughly rinsed with non-bacteriostatic saline before specimens are obtained [68]. Cornea scraping or biopsy of lesion tissue is optimal to isolate bacteria, mycobacteria, fungi, and amoeba. Swabs are acceptable for molecular detection of viral etiologies but are suboptimal for isolation of other pathogen groups.

In the industrialized world, the most common predisposing factor for the development of infectious keratitis is improper use or contamination of contact lens (CL) systems. Common risk factors include: sleeping and swimming with CL, poor hygiene and using extended wear lenses beyond recommended time intervals [117, 118]. Lens care solutions and cases exhibit transient colonization by environmental bacteria, commensal yeasts, molds, mycobacteria, and amoeba but only a subset of these microbes cause keratitis and they should not be cultured given high false positive rates [68]. *P. aeruginosa* is the most common cause of CL-associated keratitis but CL-related outbreaks due to both *Fusarium* and *Acanthameoba* are well described [68, 119, 120].

Trauma is a major route of cornea infection in agricultural settings within industrialized nations and a significant cause of blindness in developing nations [68]. It is most commonly caused by environmental pathogens including *P. aeruginosa, Nocardia* spp, nontuberculous mycobacteria, and molds including *Fusarium* spp, *Aspergillus* spp, *Curvularia* spp, and other dematiaceous fungi [68]. Additional rare causes of keratitis include: microsporidia, free-living *Hartmannella* and *Vahlkamphid* amoeba, *Prototheca* algae, and *Pythium* oomycetes [121, 122].

Post-surgical keratitis is not common but the outcome can be devastating with significant permanent vision loss [123, 124]. Commonly encountered organisms include: *S. aureus, S. pneumoniae, C. acnes,* CNS, viridans streptococci, β-hemolytic streptococci, diphtheroids, *P. aeruginosa, Serratia marcescens, Moraxella* spp, *Candida albicans*, and *Aspergillus* spp. [124-127]. *Bacillus cereus* ocular infections are particularly aggressive and considered a medical emergency [68]. *Mycobacterium chelonae* outbreaks are well described and associated with contaminated water sources [68, 128]. Given the lack of antifungal agents in cornea holding medium, the majority (~80%) of keratitis post-corneal transplantation is caused by *Candida* spp

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[129]. Keratitis also results from HSV and VZV reactivation and is a well-recognized and devastating complication of vaccinia (cowpox) vaccination [68, 130].

Endophthalmitis

Table 15 highlights the most common pathogens causing endophthalmitis, panophthalmitis, uveitis, and retinitis. All cases of endophthalmitis should be considered potential sight-threatening medical emergencies and require prompt diagnostic work-up and treatment. Endophthalmitis is characterized by inflammation within the vitreous [131], and is remarkable for its severity. Symptoms include: pain, decreased vision, eyelid and/or corneal edema, conjunctival chemosis and injection, vitritis, and /or hypopyon (a microscopic, but sometimes grossly visible, pool of leukocytes within the anterior chamber). It can be caused by direct extension of a local infection (exogenous) or seeding from the bloodstream (endogenous). Most specimens are small volume and test prioritization is critical [131, 132]. Vitrectomy procedures collect large fluid volumes (> 5ml) by "washing" the vitreous with non-bacteriostatic saline and may require concentration to optimize yield [68].

Exogenous. Exogenous endophthalmitis is caused by extension of superficial ocular or periocular infection into the eye, penetrating trauma by foreign objects, or post-surgical procedures or intravitreal injection [68, 131]. The majority (~70%) are associated with recent eye surgery including cataract, LASIK, keratoplasty, trabeculectomy and glaucoma drainage implants and most often caused by gram-positive bacteria including CNS and *C. acnes* [68, 129, 131]. Endophthalmitis caused by foreign objects is typically due to commensal skin microbiota such as CNS, *Streptococcus* spp, *S. aureus,* and *Bacillus* spp, as well as environmental organisms such as *P. aeruginosa, Nocardia* spp, and mycobacteria [68, 131, 133]. Trauma with plant material increases the likelihood of mold infection [131, 134]. Intravitreal drug injections, such as humanized monoclonal antibodies to treat macular degeneration and diabetic retinopathy, are increasingly more common and associated with outbreaks due to various glucose non-fermenters including *P. aeruginosa*, as well as *Enterobacterales,* and molds [68, 135]. Extension of infection into the posterior eye from adjacent tissue is most commonly due to the same organism previously isolated from the periocular sample. However, culture, molecular, or serologic analysis of vitreous fluid is the most direct approach to identify the infectious cause of exogenous endophthalmitis.

Endogenous. Endogenous endophthalmitis is the result of hematogenous seeding of bacteria, fungi, viruses, and parasites [136]. Risk factors include: indwelling venous catheters, immunosuppression, intravenous drug use, diabetes, and a history of disseminated infection [136, 137]. Causative agents parallel the most common causes of bloodstream infection including *Candida albicans* and related yeasts, endemic fungi, *S. aureus, S. pneumoniae, P. aeruginosa,* and Enterobacterales bacteria, particularly *Klebsiella pneumoniae* [68]. Any patient with signs and symptoms of endogenous endophthalmitis should be evaluated for current or prior systemic infection. Although, a history of prior systemic infection significantly increases the likelihood of

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the same organism causing posterior eye infection, definitive identification necessitates culture, molecular, or serologic analysis of vitreous fluid.

Panophthalmitis. Panophthalmitis is a medical emergency and can involve*: S. aureus,* β-hemolytic streptococci*, B. cereus, Clostridium* species *N. meningitidis, P. aeruginosa,* and *K. pneumoniae*, particularly hyperviscous isolates [68]. Inflammation involves the entire eye including the sclera and the adjacent extraocular tissues often resulting via direct extension from peri-ocular tissues or seeding from the blood stream [68]. Symptoms include: severe eyelid edema, conjunctival chemosis, proptosis, fixed pupil, and limited ocular movement. If scleral involvement is substantial, thinning and perforation may occur resulting in loss of the eye. Although enucleation or evisceration may be prevented with prompt initiation of antimicrobials and steroids, the prognosis for recovery of sight is dismal [68].

Uveitis/retinitis

The inflammation characteristic of uveitis/retinitis is typically due to either autoimmune conditions or is idiopathic. Only infrequently is it due to infection which is almost always caused by endogenous microbes accessing the eye via a breach in the blood-eye barrier. Because uveitis and retinitis, like endogenous endophthalmitis, are localized manifestations of systemic infections, diagnosis of the etiology of systemic infections should be coupled with a careful ocular examination performed preferably by an ophthalmologist with specific infectious disease expertise. Definitive identification of infectious causes of anterior uveitis, intermediate uveitis, and pan-uveitis requires culture, molecular, or serologic analysis of aqueous humor from the anterior chamber. Similar analyses on vitreous humor from the posterior chamber are optimal to identify microbial causes of posterior uveitis. Important causes of uveitis/retinitis include *Toxoplasma gondii*, cytomegalovirus, HSV, VZV, *Mycobacterium tuberculosis*, and *Treponema pallidum* [68].

Uveitis. Uveitis is inflammation of any portion of the uveal tract composed of the choroid, iris, and ciliary body and is categorized anatomically as either: anterior uveitis - the anterior chamber is the primary site of inflammation; intermediate uveitis - involves primarily the peripheral retina with overlying vitreous inflammation and posterior uveitis, which principally involves the posterior retina, vitreous body, and/or choroid. In pan-uveitis, all uveal structures are involved. Symptoms include eye redness, pain, photophobia, blurred vision, and floaters [138]. Uveitis can be caused by trauma, autoimmune disorders, neoplasia, idiopathic inflammation, and infection [138]. 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. *Toxoplasma gondii,* CMV, HSV, and VZV are the most common causes [138]. Rare viral causes include: West Nile, Dengue, Chikungunya, Rift Valley, Zika, and Ebola [68]. Uveitis can be caused by arthropod-borne *Borrelia* spp and *Rickettsia* spp [139]. Ocular syphilis is increasing in men who have sex with men [68, 140, 141] and *M. tuberculosis* uveitis remains a

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major global health issue [78, 142] Rubella and *Toxocara* spp should also be considered in pediatric patients [68].

Retinitis Retinitis is inflammation of the neurosensory retina. Symptoms include photophobia, blurred vision, ocular pain, and floaters. *T. gondii* and CMV are the most common etiologic agents [143, 144]. Toxoplasmosis accounts for ~ 90% of all focal necrotizing retinitis. It is characterized by a "headlight in the fog" retinal lesion, and can be diagnosed as described above using a combination of NAAT and intra-ocular serology [68]. CMV retinitis is frequently diagnosed clinically because of characteristic lesions seen on ophthalmologic examination [68]. Patients with detectable CMV viral loads have a higher likelihood of retinal disease progression [145]. 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 [146]. Physicians should consult the laboratory performing the CMV viral load for assistance with test interpretation.

Table 13. Laboratory Diagnosis of Pre-Septal and Orbital Cellulitis, Lacrimal and Eyelid Infections, and Conjunctivitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria			
Streptococcus pneumoniae *Staphylococcus aureus* *Streptococcus pyogenes* *Pseudomonas aeruginosa* *Escherichia coli* Other Enterobacterales *Moraxella catarrhalis* *Haemophilus influenzae* *Neisseria gonorrhoeae*	Gram stain Aerobic bacterial culture	Swab of purulent discharge	Swab transport device, Room temperature (RT), 2 hr
Actinomyces spp Other anaerobic bacteria (rare cause of canaliculitis)	Anaerobic bacterial culture	Expressed concretions, scraping or biopsy	Sterile anaerobic container, RT, immediately
Chlamydia trachomatis	NAAT [1] Direct fluorescent antibody stain	Swab of purulent discharge	Virus swab transport device, RT, 2 hr
Fungi			
Candida spp	Aerobic bacterial or fungal culture	Swab of purulent discharge	Swab transport device, RT, 2 hr
Mucorales molds *Aspergillus* spp *Fusarium* spp	Fungal culture	Sinonasal tissue [2]	Sterile container, RT, 2hr
Microsporidia	Direct exam, calcofluor white, modified trichrome	Biopsy of lesional tissue, typically conjunctiva	Sterile container, RT, 2hr
Viruses			

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Herpes simplex virus (HSV) Varicella zoster virus (HSV) Herpes B virus [4]	HSV NAAT [3] VZV NAAT [3]	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Adenovirus	NAAT [5] or antigen test	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Enterovirus, Coxsackie virus, Arboviruses, Ebola	NAAT	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Parasites			
Toxocara canis and other helminth infections	Direct exam [6]	Removal, Biopsy	Sterile container, RT, 2hr

Abbreviations- (RT=Room Temperature):

[1] NAATs for detection of *C. trachomatis* are more sensitive than DFA but have not yet been approved in the United States for use with conjunctival swab specimens. Laboratories that offer such "off label" testing must conduct in house validation of this specific specimen type prior to use in patient care.

[2] Invasive oculosinonasal mold infections have high morbidity and mortality. If suspected it is critical for providers to notify the lab as mincing (not grinding) the tissue is critical to ensure viability of hyphae and optimize culture yield.

[3] HSV and VZV culture is only indicated in refractory cases with high suspicion for antiviral resistance.

[4] Culturing Herpes B virus requires biosafety level 4 precautions. Consult the lab when this entity is suspected. Exposure risks include being bitten or scratched by an infected monkey or contact with the monkey's eyes, nose, or mouth. Diagnostic testing requires coordination with the National B virus Resource Center at Georgia State University.

[5] Adenovirus NAATs are more sensitive but given high viral burden, antigen tests also exhibit high sensitivity.

[6] Worm extraction is required for diagnostic and therapeutic purposes. Adult *Loa loa, Thelazia, Dirofilaria*, and *Toxocara* spp nematodes cause conjunctivitis and can be identified by morphology. NIH and CDC diagnostic labs also offer specialized molecular testing.

Table 14. Laboratory Diagnosis of Infectious Keratitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria [1]			
Coagulase negative staphylococci *Staphylococcus aureus* *Streptococcus pneumoniae* *Corynebacterium* spp *Cutibacterium acnes* *Nocardia* spp [3] *Pseudomonas aeruginosa* *Serratia marcescens* *Escherichia coli* Other Enterobacterales *Haemophilus influenzae* *Neisseria gonorrhoeae*	Gram stain [2] Aerobic bacterial culture Add BCYE agar for *Nocardia*	Corneal scrapings, biopsy	Room temperature (RT), immediately
	Anaerobic culture (for *C. acnes*)	Corneal scrapings, biopsy	Place second sample into anaerobic broth
Mycobacterium spp [4]	Acid fast smear AFB culture	Corneal scrapings, biopsy	Sterile container, RT, 2 hr
Fungi			

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Candida spp	Aerobic bacterial or fungal culture	Corneal scrapings, biopsy	Swab transport device, RT, 2 hr
Aspergillus spp *Fusarium* spp *Curvularia* spp Dematiaceous fungi	Calcofluor white-KOH stain[5] Fungal culture[5]	Corneal scrapings, biopsy	RT, immediately[5]
Microsporidia	Direct exam, calcofluor white, modified trichrome	Biopsy	Sterile container, RT, 2hr
Viruses			
Herpes simplex virus (HSV)	HSV NAAT (for initial diagnosis)	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Varicella zoster virus (VZV)	VZV NAAT	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Adenovirus	Adenovirus NAAT or antigen test	Swab of lesional tissue	Viral swab transport device, RT, 2 hr
Enterovirus/Coxsackie virus	NAAT	Swab of lesional tissue	Virus swab transport device, RT, 2 hr
Parasites			
Acanthamoeba spp	Calcoflour-KOH stain Giemsa stain[6]	Corneal scrapings, biopsy	Plastic slide holder, RT, immediately
	Acanthamoeba NAAT (most sensitive) or culture[7]	Corneal scrapings, biopsy, swab of lesional tissue (for NAAT only)	RT, immediately, Swab/tissue for NAAT in Universal transport media or saline, RT, 2 hr
Onchocerca volvulus	Direct exam[8]	Biopsy of skin nodule	Sterile container, RT, 2hr

Abbreviations- (RT=Room Temperature):

[1]The relative likelihood of a specific etiology depends on the underlying cause (trauma, post-operative, contact lens-related).

[2] As indicated, smear scraping material onto a demarcated area on a clean glass slide and transport to the lab for Gram stain, calcofluor white, or direct fluorescent antibody staining. Transport of slides using plastic slide holder helps mitigate the introduction of calcofluor-white positive artifacts, including cardboard material.

[3]The lab should be notified when *Nocardia* spp is suspected so that incubation is extended and additional media, such as buffered charcoal yeast extract, utilized.

[4]Acid fast smears and mycobacterial cultures should be performed in all post-operative infections to identify rapidly growing mycobacteria including *Mycobacterium chelonae*.

[5]Inoculate cornea scrapings onto at least one culture plate containing a non-selective fungal growth medium, such as potato dextrose agar, at the bedside. If sufficient sample is available, smear scraping material onto a glass slide as described above to evaluate for fungal elements.

[6]For direct microscopic detection of *Acanthamoeba*, cornea scraping material should be inoculated directly onto a demarcated area on a clean glass slide and transported to the lab in a plastic slide holder as outlined above.

[7] If *Acanthamoeba* culture is available in-house, cornea scrapings should be sent to the lab for processing. In the lab, the sample is seeded onto a lawn of viable *E. coli* on non-nutritive agar or other Enterobacterales bacteria (i.e., co-cultivation) and incubated enabling visualization of characteristic tracks, cysts, and trophozoites by microscopy. If the sample will be shipped to a reference lab or a delay in processing is anticipated, immersion of the sample in Page's amoeba saline is recommended.

[8] Diagnosis typically made via FFPE evaluation of gravid adults in skin nodules.

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Table 15. Laboratory Diagnosis of Endophthalmitis, Panophthalmitis, Uveitis, and Retinitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria [1]			
Coagulase negative staphylococci *Staphylococcus aureus* *Streptococcus agalactiae* Viridans streptococci *Enterococcus* spp *Bacillus cereus* *Cutibacterium acnes* *Corynebacterium* spp *Listeria monocytogenes* *Nocardia* spp [2] *Pseudomonas aeruginosa* *Serratia marcescens* *Acinetobacter* spp *Escherichia coli* Other Enterobacterales *Haemophilus influenzae* *Neisseria meningitidis*	Gram stain Aerobic bacterial culture Add BCYE agar for *Nocardia*	Aqueous aspirate Vitreous aspirate, washing or biopsy	Transport directly to the lab , Room temperature (RT), immediately Washing sent to lab @ RT, 2hr
	Anaerobic culture for *C. acnes*	Place second sample into anaerobic broth	Sterile anaerobic container, RT, immediately
Treponema pallidum	VDRL	CSF	RT, 2 hr
Borrelia burgdorferi	Serology	Serum	RT, 2 hr
Mycobacterium spp[3]	Acid fast smear AFB culture	Aqueous aspirate Vitreous aspirate, washing or biopsy	Inoculated slants and smear are transported directly to the lab, RT, immediately Washing sent to lab @ RT, 2hr
Viruses			
Herpes simplex virus (HSV) Varicella zoster virus (VZV)	HSV NAAT VZV NAAT	Swab	RT, 2 hr
Adenovirus	NAAT or antigen test	Swab	RT, 2 hr
Enterovirus, Coxsackie virus, Arboviruses, Ebola [4]	NAAT	Swab	RT, 2 hr
Fungi [5]			
Candida albicans *Nakeseomyces (Candida) glabrata* Other *Candida* spp *Aspergillus* spp *Fusarium* spp Dematiaceaous fungi Dimorphic molds	Calcofluor-KOH stain Fungal culture	Aqueous aspirate Vitreous aspirate, washing or biopsy	RT, immediately Washing sent to lab @ RT, 2hr
Parasites			

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Toxoplasma gondii	Serology or NAAT [6]	Ocular fluid	RT, 2 hr
Toxocara spp, *Taenia solium*, *Echinococcus*, and other helminths infections	Direct exam [7]	Removal, Biopsy	Sterile container, RT, 2hr

Abbreviations- (RT=Room Temperature):

[1] 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. The other bacteria listed may additionally cause endophthalmitis either secondary to trauma or surgery.

[2] The lab should be notified when *Nocardia* spp is suspected so that incubation is extended and additional media, such as buffered charcoal yeast extract, utilized

[3] The most common *Mycobacterium* spp recovered from intraocular infections is *M. chelonae* and this occurs almost exclusively as a complication of surgical procedures. Acid fast smears and mycobacterial cultures should be performed in all post-surgical infections.

[4] Ebola will most often present in the context of a characteristic clinical syndrome. In this setting, viral detection and/or serologic confirmation in other specimen types (usually blood) is sufficient evidence for ocular involvement. If suspected, rapid communication to public health partners and appropriate infection prevention measures is indicated.

[5] Yeasts and dimorphic molds cause endogenous endophthalmitis via hematogenous spread and eye exams by trained ophthalmologists are critical in patients with documented fungemia. Molds additionally gain entry to the back of the eye via direct extension from the ocular surface or periocular cavities including traumatic inoculation and intraocular injection.

[6] Chronic *Toxoplasma gondii* ocular infection is diagnosed via intra-ocular serology coupled with clinical findings, however, when antibody levels are low and organism burden is high, such as early acute infection or immunocompromised states, NAATs exhibit higher sensitivity.

[7] Helminth extraction is required for diagnostic and therapeutic purposes. Compatible serology can help render the diagnosis. NIH and CDC diagnostic labs also offer specialized molecular testing.

VI. 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, and maceration due to excessive moisture, poor blood perfusion, or other factors that disrupt the stratum corneum. SSTIs are often classified as simple (uncomplicated) or complicated (necrotizing or non-necrotizing) and may involve skin, subcutaneous fat, fascial layers, and musculotendinous structures [147]. Thus, any compromise of skin and skin structure provides a point of entry for a myriad of exogenous and endogenous microbial microbiota that can produce a variety of infections. Infections of the skin and soft tissue are often characterized as pyodermas, infections associated with underlying conditions of the skin, and necrotizing infections. Simply stated, SSTIs range from simple superficial infections to severe necrotizing infections. Representative primary cutaneous infections or uncomplicated pathological conditions include cellulitis, ecthyma, impetigo, folliculitis, furunculosis, and erysipelas and are commonly caused by a narrow spectrum of pyogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes* (Group A streptococcus). Secondary infections are often extensions of pre-existing lesions (traumatic or surgical wounds, and ulcers) which serves 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.

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Anaerobic bacteria are important and predominate pathogens in DFIs and should always be considered when choosing therapeutic options. The majority of DFIs are polymicrobial but Gram-positive cocci, specifically staphylococci, are the predominate infectious agents; *Pseudomonas aeruginosa* is a major pathogen in the majority of chronic DFIs. Surface cultures of such wounds, including decubitus ulcers are of little value as they represent colonizing microbes which cannot be easily differentiated from the underlying etiologic agent. Tissue biopsies, preferably a full-thickness biopsy, following thorough debridement, or bone biopsies obtained through a debrided site, are optimal for obtaining clinically relevant information [148, 149]. Necrotizing cutaneous infections, such as necrotizing fasciitis, are usually caused by streptococci and less often by methicillin-resistant *S. aureus* (MRSA) or *Klebsiella* species but are often 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 lesion or tissue 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 [150].

A major and most important factor in acquiring clinically relevant results is to ensure the acquisition of appropriate specimens that represent the disease process. Guidelines or critical points for obtaining representative specimens are summarized as follows:

In this section, cutaneous infections, involving skin and soft tissue, have been expanded and categorized as follows: trauma-associated, surgical site, burn wound, fungal, viral, human and animal bites, and device-related. Although *S. aureus,* especially MRSA, and *S. pyogenes* are the causative agents in the majority of these infections, other microorganisms, including fungi and viruses, are important and require appropriate medical and therapeutic management. It is also important for the ordering clinician to be familiar with the level of services provided by the supporting laboratory. For example, few laboratories perform quantitative cultures for the assessment of wounds, especially burn wounds, or 16S ribosomal RNA gene PCR/sequencing (Sanger and/or next-generation sequencing [NGS]), or other molecular diagnostics which have high sensitivity compared to culture-based methods. NGS (16S and metagenomic) has gained popularity in the orthopedic surgery community in support of their efforts to diagnose prosthetic joint infections (PJI) by testing joint fluid, bone, or multiple samples from the margin of the infected site [150]. Only a limited number of academic medical centers and commercial reference laboratories offer 16s RNA sequencing and/or NGS. If a clinically indicated service or procedure is not available in the local microbiology laboratory, consult with the Laboratory Director so that arrangements can be made to outsource the specimen to a qualified licensed reference laboratory with the understanding that turn-around-times (TAT) are usually longer thus extending the time to

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receipt of results. At minimum, a Gram stain should be performed to assess and characterize indicators of surface contamination (squamous epithelial cells) or infection (white blood cells) combined with a description of morphotypes (i.e. Gram-positive cocci in chains, pairs, or clusters, Gram-negative rods, Gram-positive bacilli, Gram-negative small, pleomorphic bacilli). Mass spectrometry, i.e., MALDI-TOF, has replaced conventional biochemical identification for the identification of bacteria, mycobacteria, and fungi. The data bases within these systems are expansive and it is not unusual for reports to contain organisms that may be unfamiliar to many clinicians. These organisms can pose a challenge to both the provider and microbiologists regarding clinical relevance. Conducting an extensive literature search is a productive way of gleaning information that should be shared with providers, infectious diseases practitioners, pharmacists, and infection control and prevention personnel.and decide if those organisms should be reported or considered part of the microbiota of the skin.

Key points for the laboratory diagnosis of skin and soft tissue infections

- A swab is not the optimal choice for these specimens. Submit tissue, fluid, aspirate when possible.
- Do not use the label "wound" alone. Be specific about the actual anatomical site and type of wound (i.e. "human bite wound from the right forearm", "surgical incision") from which the specimen was collected.
- The specimen of choice is a firm sample of the advancing margin of the wound/lesion, not just the surface of the wound/lesion.
- Pus alone is inadequate and does not specifically represent the disease process.
- When submitting tissue or biopsies for culture, also request histopathology analysis. Place specimen in formalin for histopathology analysis only.
- Do not request the laboratory to report everything that grows.

Burn wound infections

Reliance on clinical signs and symptoms alone in the diagnosis of burn wound infections is difficult and unreliable. Sampling of the burn wound by either surface swab or preferably tissue biopsy for culture and histopathology is recommended for monitoring the presence and extent of infection (Table 16). Quantitative culture of either specimen is recommended; optimal utilization of 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 microbiota on the surface of the wound rather than the advancing margin of the subcutaneous or deep, underlying damaged tissue. Swabs have several limitations, (a) higher risk of contamination with surface and subsurface microbiota; (b) limited volume capacity (25 – 50 ul) leading to insufficient quantity of specimen especially when cultures other than bacteriology are requested (fungal and mycobacterial). Bacterial culture of tissue biopsy should always be supplemented with histopathology examination to better ascertain the extent of microbial invasion. Prior to collecting surface samples or tissue biopsy (ensure that separate tissue specimens be

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collected, one for culture, and one placed in formalin for histopathology, the wound should be thoroughly cleansed and devoid of topical antimicrobials that can affect culture results. Blood cultures should also be obtained for the detection of systemic disease secondary to the wound.

The application of nucleic acid amplification tests (NAAT) for the detection of viruses is commonly restricted to blood and/or body fluids (vesicular, infected fluids). The provider should be assured that the laboratory has validated such assays and if the laboratory has assessed the performance of the assay for tissue specimens. This precaution would also apply to the molecular detection of MRSA in SSTIs and vancomycin resistant enterococcus (VRE); this would also apply to the commercially available multiplex molecular platforms [151, 152].

Table 16. Laboratory Diagnosis of Burn Wound Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			
Staphylococcus aureus Coagulase-negative staphylococci *Enterococcus* spp *Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae* *Serratia marcescens* *Proteus* spp *Aeromonas hydrophila*[2] *Bacteroides* spp and other anaerobes	Aerobic culture	Tissue (punch biopsy) Aspirate Blood culture Surface swab	RT, <12 h, aerobic RT, <2 h, transport medium No formalin, keep moist
	Histopathology	Tissue (punch biopsy)	Submit in formalin RT, 2 h
	Anaerobic culture	Tissue biopsy or aspirate (swab may not represent the disease process)	Anaerobic transport tubes, pre-reduced media; RT, <2 h
		Swab from manufacturer collection kit	Laboratory-provided transport device, RT, <2 h
Fungi			
Candida spp *Aspergillus* spp *Fusarium* spp *Alternaria* spp Zygomycetes	Fungal culture	Tissue biopsy	RT, < 30 min, no formalin, keep moist
	Fungal blood culture	Blood; 2-4 blood culture sets per 24 h period	Broth-based bacterial (aerobic) or fungal blood culture bottles, RT, <2 h
Viruses			
Herpes simplex virus Cytomegalovirus Varicella-zoster virus	Tissue culture or NAAT	Tissue (biopsy/aspirate)	Viral transport medium or laboratory-provided transport device

[2]Electrical burns, potential for transmission from leeches

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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. Relevant pathogens to consider are: *Eikenella, S. aureus,* group A *Streptococcus, Fusobacterium, Peptostreptococcus, Prevotella, and Porphyromonas* spp, in addition to human skin microbiota (staphylococci, streptococci). Other less common bacterial pathogens include the members of the HACEK group (*Aggregatibacter aphrophilus* and *Actinobacillus actinomycetemcomitans; Capnocytophaga, Cardiobacterium, Eikenella,* and *Kingella spp* Viral pathogens of importance include Hepatitis B, Hepatitis C, HIV, and Herpes Simplex Virus [153-156]. Such infections are common in the pediatric age group and are often inflicted during play or by abusive adults. Clenched-fist injuries and occlusal bites are usually associated with 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 abscesses, sepsis with accompanying disseminated intravascular coagulation (DIC), especially in immunocompromised patients.

For patients with clinically uninfected bite wounds, cultures are not indicated due to the lack of correlation with subsequent infection. In the presence of clinical signs of infection, the challenge is to acquire a representative specimen for aerobic and anaerobic culture with the major limitation of culture being the potential for generating misleading information due to 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 for reasons previously mentioned (see Burn Wounds); aspirate and/or tissue is preferred for culture and Gram stain (Table 17).

Table 17. Laboratory Diagnosis of Human Bite Wound Infections

Etiologic Agents	Diagnostic Procedures[1]	Optimum Specimens	Transport Issues
Bacterial			
Aerobes Mixed aerobic and anaerobic oral microbiota	Aerobic/anaerobic culture Gram stain	Tissue Biopsy/aspirate	Anaerobic transport conditions/vials

[1] No utility in collecting a specimen at the time of the bite; collect samples only if infection is suspected or likely.

Animal bite wound infections

As with human bite wounds, the oral cavity is the primary source of potential pathogens and thus the anticipated etiological agent(s) is highly dependent upon the type of animal that inflicted the bite (Table 18). Since dogs and cats account for most animal-inflicted bite wounds, the two most prominent microorganisms that should be initially considered in the evaluation of patients are

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Pasteurella spp, namely *P. canis* and *Capnocytophaga canimorsus* (dogs), and *P. multocida* subspecies *multocida* and *septica* (cats). Other common aerobes include streptococci, staphylococci, *Moraxella* spp and saprophytic *Neisseria* spp It is not uncommon for animal bite wounds to be polymicrobial in nature which includes as a variety of anaerobes. Due to the complexity of the microbial microbiota in animals, examination of cultures for organisms other than those listed in Table 18 is of little benefit since these organisms are not included in most of the commercial identification systems (conventional and automated), and most likely, MALDI-TOF databases [157-166]. If rabies or Herpes B infection is suspected, contact the local or state health department for assistance and guidance on how to proceed.

Although rat-bite fever, caused by *Streptobacillus moniliformis*(most common in US) or *Spirillum minus*(most common in Asia), is rare, it can be transmitted to humans in up to 10% of rat bites (www.cdc.gov). The organism is commonly present in the oropharynx of rats and other rodents.

Table 18. Laboratory Diagnosis of Animal Bite Wound Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial[1]			
Actinobacillus spp *Capnocytophaga* spp *Erysipelothrix rhusiopathiae* *Pasteurella* spp *Streptobacillus* spp	Aerobic/anaerobic culture Gram stain	Tissue/biopsy/aspirate blood, synovial fluid, or other body fluids	Anaerobic transport container[2] Be certain to provide sufficient volume of sample for complete culture and Gram stain evaluation; RT, <2 h
	Blood culture	Blood; 2-4 blood culture sets per 24 h	Blood culture bottles, RT, <2 h
	Aerobic culture	Tissue/biopsy/aspirate	Sterile container

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Mycobacterium fortuitum *M. kansasii*	Acid-fast culture Acid-fast stain		RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Transport in formalin, RT, 2 h-24 h

[1]Additional potential pathogens to consider: Staphylococcus intermedius, Bergeyella zoohelcum, –Cutibacterium (Propionibacterium) spp, Filifactor spp, Moraxella spp, Neisseria spp, Kingella spp, Pseudomonas fluorescens, Halomonas venusta, CDC Group EF-4, CDC NO-1, Peptococcus spp,
Rabies, Herpes B, or other viruses (refer to Viral Section XVI);
[2]Anaerobic transport media preserve all other organisms for culture

Trauma-associated cutaneous infections

Infections secondary to trauma are usually caused by exogenous or environmental microbial microbiota but can be due to the individual's indigenous (normal) microbiota. It is strongly recommended that specimens be submitted for culture at a minimum of 48 hours post-trauma since growth from specimens collected within the first 48 hours most likely represents environmental microbiota 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 [167-170]. It is strongly recommended that initial cultures focus on common pathogens with additional testing (including molecular-based diagnostics) being reserved for uncommon or rare infections associated with special circumstances (ex: detection of *Vibrio* spp following saltwater exposure or patients with chronic manifestations of infection who do not respond to an initial course of therapy).

Although not considered in the same manner as external trauma, people who inject drugs (PWIJ) inject themselves with exogenous substances that may include spores and other contaminants that cause SSTIs ranging from abscesses to necrotizing fasciitis. Agents like those listed in Table 19, with the addition of *Clostridium sordelli* and *C. botulinum* (wound botulism), and the agents of human bite wounds (Table 17) among subcutaneous injectors who use saliva as a drug diluent.

Table 19. Laboratory Diagnosis of Trauma-Associated Cutaneous Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			

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Staphylococcus aureus Group A, B, C, and G streptococci *Aeromonas hydrophila* and other *Aeromonas* spp *Vibrio vulnificus* *Bacillus anthracis*[2] *Clostridium tetani*[3] *Corynebacterium* spp Mixed aerobic/anaerobic microbiota (cutaneous origin)	Aerobic/anaerobic culture NAAT[1] Blood culture	Surgical tissue aspirate Blood	Aerobic/anaerobic conditions or anaerobic transport device; keep tissue moist Aerobic/anaerobic blood culture bottles, RT, <2 h
	Histopathology	Surgical tissue Biopsy/aspirate	Formalin container, RT, <24 h
Mycobacterium spp *Nocardia* spp	Mycobacterial culture Nocardia culture Acid-fast smear	Tissue/ aspirate	Sterile container, RT, <2 h
	Histopathology	Tissue/ /aspirate	Formalin container, RT, <24 h
Fungal			

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Aspergillus spp Mucorales Zygomycetes Dematiaceous moulds *Sporothrix schenckii* *Histoplasma capsulatum* *Blastomyces dermatitidis* *Coccidioides immitis* *Talaromyces (Penicillium) marneffei* Yeasts (*Candida* spp including *C. auris* /*Cryptococcus* spp) Other filamentous fungi	Fungal culture Calcofluor-KOH preparation	Surgical tissue aspirate	Aerobic transport device Keep tissue moist; avoid formalin fixation
	Histopathology	Surgical tissue aspirate	Formalin container, RT, 2 h – 24 h

[1]There is an FDA-cleared NAAT for direct detection of *S. aureus* and MRSA from swabs of wounds and pus
[2] Select agent: if suspected, notify laboratory in the interest of safety
[3]*Clostridium tetani* can also be an etiological agent of trauma-associated infections in rare cases. This is usually a clinical diagnosis rather than a laboratory diagnosis.

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Surgical site infections

Surgical site infections (SSIs) may be caused by endogenous microbiota or originate from exogenous sources such as healthcare providers, the environment, or material used for surgery that are commonly classified as incisional and organ/space manipulated during a surgical procedure. Incisional, especially deep and organ/space infections are SSIs that are further divided into

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superficial (skin and subcutaneous tissue) and deep (tissue, muscle, fascia). Deep incisional and organ/space SSIs are associated with the highest mortality. The reader is referred to the Centers for Disease Control and Prevention Guidelines for Prevention of Surgical Site Infections, 2017, (www.cdc.gov) for specific definitions of SSIs. Of the microbial agents listed below (Table 20), *S. aureus,* including MRSA, coagulase-negative staphylococci, and enterococci are isolated from nearly 50% of these infections [171]. During surgical procedures, do not collect swabs and instead, submit tissue, fluids, or aspirates. Although enterococcal species are commonly isolated from superficial cultures, they are seldom true pathogens; and therapeutic regimens that do not include coverage for enterococci are usually successful in treating surgical infections. The recommended IDSA therapeutic regimens for SSIs, are not reliably active against these organisms [148].

Table 20. Laboratory Diagnosis of Surgical Site Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			
S. aureus Coagulase-negative staphylococci Beta-hemolytic streptococci (Group A, B, C and G) Alpha- and Non-hemolytic streptococci Enterococci *Acinetobacter* spp *Pseudomonas aeruginosa*	Gram stain Aerobic culture and AST NAAT[1]	Tissue/biopsy/aspirate	Keep tissue moist; aerobic transport, RT, <2 h
	Anaerobic culture Suspicion for flesh eating streptococci or abscess	Tissue/biopsy/aspirate	Anaerobic transport device RT, <2 h
	Blood culture	Aerobic and anaerobic bottles	RT, <2 h

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Enterobacteriaceae	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2 h – 24 h RT, indefinite
Mycoplasma hominis and *Legionella pneumophila* (rare but possible agents in specific situations)[2]	Culture (mycoplasma culture requires special handling)	Tissue/biopsy/aspirate	Special transport medium; check with laboratory if available
Mycobacterium spp-rapid growers	Acid-fast stain and *Mycobacterial* culture	Tissue/biopsy/aspirate	Aerobic transport device Sterile container RT, <2h
Fungi			
Candida spp	Fungal culture Calcofluor-KOH preparation	Tissue/biopsy/aspirate	Aerobic transport device Sterile container RT, <2h
	Fungal blood culture	Blood	Lysis-centrifugation blood culture tube or aerobic blood culture bottles, RT, <2h

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	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2 h – 24 h

[1]There is an FDA-cleared NAAT for direct detection of *S. aureus* and MRSA from swabs of wounds and pus
[2]*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.

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 cave filters, vertebroplasty, nephrostomy placement, radiologically inserted gastrostomy, dialysis access and related intervention, transjugular intrahepatic proto-systemic shunt, biliary intervention, and endovenous laser ablation of varicose veins) performed using image guidance. Procedures are regarded as either diagnostic, (e.g., angiogram) or performed for treatment purposes, (e.g. angioplasty). Images are used to direct procedures that are performed with needles or other tiny instruments (e.g. catheters). The images are analogous to a road map that allows the radiologist to guide these instruments through the body to the intended areas of concern. 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 (e.g., 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, amount of suction required, 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 contaminate a drain or its tubing typically depend on the anatomic location and position of the drain (superficial,

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intraperitoneal, or within an organ, duct, or fistula) and the indication for its use. Ideally, submit drain specimens soon after insertion since interpretation of culture results from drains that have been in place for > 3 days may be difficult due to the presence of colonizing bacteria and yeast.

Drains are characterized as gravity, low-pressure bulb evacuators, spring reservoir, low pressure or high pressure. Fluids from drains are optimal specimens for collection and submission to the microbiology laboratory. All fluids should be collected aseptically and transported to the laboratory in an appropriate transport 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 microbiota represents the predominate potential pathogens.

Cutaneous fungal infections

The presence of fungi (molds or yeasts) on the skin poses a challenge to the clinician in determining if this represents contamination, saprophytic colonization, or is a true clinical infection. Consideration should be given to *Candida auris,* a yeast that has recently gotten much attention due its causing skin infections and resistant to most antifungal agents. For convenience, the fungi are listed by type of mycosis they commonly produce (Table 21). Dermatophytes typically produce tinea (ringworm)-type infections; dematiaceous fungi (darkly pigmented molds and yeast-like fungi) cause both cutaneous and subcutaneous forms of mycosis; dimorphic fungi generally cause systemic mycosis and the presence of cutaneous lesions signifies either disseminated or primary (direct inoculation) infection, yeast-like fungi are usually agents of opportunistic-type mycosis but can also manifest as primary or disseminated disease as is true for the opportunistic molds (e.g *Aspergillus* spp, *Fusarium* spp). In addition to the recommended cultures and associated optimal specimens, fungal serology testing (complement fixation and immunodiffusion performed in parallel) are often beneficial in diagnosing agents of systemic mycosis, specifically *Histoplasma, Blastomyces,* and *Coccidioides*. In cases of active or systemic histoplasmosis and blastomycosis, the urine antigen test may be of value in identifying disseminated disease. Only genus-level identification of dermatophytes is necessary in most cases.

The clinician should be aware that dematiaceous fungi (named so because they appear darkly pigmented-greenish brown to black on laboratory media) do not always appear pigmented in tissue but rather hyaline (clear) in nature. To account for this, a Fontana-Masson stain (histopathology) may be performed to detect small quantities of melanin produced by these fungi. While this stain is helpful to rule out dematiaceous fungi, many hyaline or dimorphic fungi may stain positive, thus this stain is not highly specific for dematiaceous fungi. It is not uncommon for this group of fungi to be mistakenly misidentified as a hyaline mold such as *Aspergillus* spp. This highlights the importance of correlating culture results with histological observation in determining the clinical relevance since the observation of fungal elements in histopathology specimens is most likely indicative of active fungal invasion [172-175].

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Table 21. Laboratory Diagnosis of Fungal Infections of Skin and Subcutaneous Tissue

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

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Aspergillus spp *Fusarium* spp Zygomycetes	Fungal culture Calcofluor-KOH preparation	Tissue/biopsy/aspirate Blood; 2 sets (*Fusarium* only)	Sterile transport container Aerobic conditions RT, <2 h Aerobic blood culture bottles or lysis/centrifugation blood cultures, RT, <2 h
	Histopathology	Tissue/biopsy/aspirate	Formalin container, RT, 2 h – 24 h

VII. UPPER RESPIRATORY TRACT BACTERIAL AND FUNGAL INFECTIONS

Infections in the upper respiratory tract involve the ears (otitis media), the mucus membranes lining the nose and throat above the epiglottis (pharyngitis), and the sinuses (sinusitis). Most infections involving the nose and throat are caused by viruses (see Section XVI for testing information). The differential diagnosis of soft-tissue infections of the head and neck frequently includes pharyngitis, otitis media, and sinusitis; those are covered in section IV. Inappropriate utilization of antibiotics for viral infections is a major driver of increasing antibiotic resistance. While much treatment of otitis media and sinusitis is empirical, management of pharyngitis, and of prolonged and refractory ear and sinus infections frequently involves laboratory tests to determine the etiology and thus inform the proper therapy.

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

- Most cases of otitis media should be diagnosed clinically and treated without culture support.
- Nasopharyngeal cultures do not accurately predict the etiologic agent of sinusitis.
- Swabs are not recommended for otitis media or sinusitis. Submit an aspirate or direct drainage collection from the involved space for culture.
- 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.

Otitis media

Otitis media (OM) is the single most frequent condition causing pediatric patients to be taken to a healthcare provider and to be given antibiotics [176]. While some children with acute otitis media (AOM) can be managed with pain relief and careful follow-up [177], in most cases antibiotic therapy is used [178, 179]. *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common bacterial causes of AOM, with *S. aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and a number of other potential pathogens

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occurring less commonly [180]. After the introduction of the 7-valent pneumococcal vaccine, and the subsequent 13-valent vaccine, the predominant pathogen identified in OM in the US changed from *S. pneumoniae* to *H. influenzae* [181]. *Alloiococcus otitidis* is also thought to be associated with AOM [180]. A variety of respiratory viruses are known to contribute to AOM, 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. Viral infections predispose to AOM due to alterations in middle-ear physiology and microbiota [182, 183], but bacterial infection of the middle-ear space remains central to pathogenesis. Chronic suppurative otitis media is associated with a higher rate of complications than acute OM. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common pathogens in chronic OM [184].

Microbiologic diagnostic work-up of otitis media 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 [176, 178, 185]. The only representative specimen is middle ear fluid obtained either by tympanocentesis or, in patients with otorrhoea or myringotomy tubes, by collecting drainage on mini-tipped 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 OM. (Table 22)

Sinusitis

Rhinosinusitis (the preferred term; encompassing both acute and chronic disease) affects approximately 12% to 15.2% of the adult population in the United States, annually. The direct costs of managing ARS and CRS exceed US$11 billion per year. In pediatrics ages 1-18, it is estimated that 6-7% of patients seeking care for respiratory symptoms had an illness consistent with rhinosinusitis (https://doi.org/10.1542/peds.2013-1071).

The etiological agents of rhinosinusitis vary based upon the duration of symptoms and whether it is community-acquired or health care associated (Table 23). *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, but most patients with acute sinusitis have an upper-respiratory virus detectable early in illness [186]. *Staphylococcus aureus*, gram-negative bacilli, *Streptococcus* spp, and anaerobic bacteria are associated more frequently with subacute, chronic or health care associated sinusitis [187]. The role of fungi as etiological agents is more controversial, possibly due to numerous publications that used poor sample collection methods and thus failed to recover the fungal agents. In immunocompetent hosts, fungi are associated most often with chronic sinusitis, though the significance of fungal presence in chronic sinusitis is frequently uncertain [186, 188, 189]. Invasive sinusitis due to fungal infections in persons with severe immunocompromise or uncontrolled diabetes is often severe and carries a high mortality rate.

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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 since an aspirate is much more productive of the true etiologic agent(s), and the normal microbiota of the nasopharynx frequently includes *Staphylococcus aureus* and other bacteria which can confuse the diagnostic picture. Endoscopically obtained swabs can recover bacterial pathogens but rarely detect causative fungi [185, 190, 191]. In maxillary sinusitis, antral puncture with sinus aspiration or, in adults only, swabs of material draining from the middle meatus obtained under endoscopic guidance, represent the only adequate specimens. Cultures of middle meatus drainage specimens in pediatric patients is controversial [188]. 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 [191].

Pharyngitis

Acute pharyngitis accounts for roughly 1.3% of outpatient visits to health care providers in the United States and was responsible for 15 million patient visits in 2006 [192]. Most pharyngitis (25-45%) is viral, and for the most part need not be treated, but 10-15% of pharyngitis in adults, and 15-30% in children, is due to group A streptococci [193]. Differences between the epidemiology of various infectious agents related to the age of the patient, the season of the year, accompanying signs and symptoms, and the presence or absence of systemic disease are insufficient to establish a definitive etiologic diagnosis on clinical and epidemiologic grounds alone [193, 194]. Consequently, the results of laboratory tests play a central role in guiding therapeutic decisions (Table 24). Antimicrobial therapy is warranted only in patients with pharyngitis with a proven bacterial etiology [195].

S*treptococcus pyogenes* (Group A beta-hemolytic *Streptococcus*) is the most common bacterial cause of pharyngitis and carries with it potentially serious sequelae (acute rheumatic fever and other complications), 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 [194]. Rapid antigen tests for *S. pyogenes*, in particular, have been used extensively in the evaluation of patients with pharyngitis. Such tests are technically non-demanding, generally reliable and often performed at the point-of care. For any of these methods, accuracy and clinical relevance depends on adequate sampling technique.

There is 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 adults have a lower risk of complications, current 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 [196]. Laboratories accredited by the

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College of American Pathologists are required to back up negative rapid antigen tests with culture according to the FDA labeling of the tests. Rapid, CLIA-waived methods for molecular group A strep testing provide improved sensitivity and may not require culture confirmation [197, 198], though they have not yet been incorporated into consensus guidelines.

Non-Group A beta-hemolytic streptococci, in particular, Groups C and G, are components of the normal pharyngeal microbiota in many cases, but are also potential causes of pharyngitis. These are most likely *Streptococcus dysgalactiae* and less frequently *Streptococcus canis* or *Streptococcus equi*. As more labs move towards identification by MALDI, Lancefield typing may not be performed. Many healthcare providers consider these organisms to be of significance and base therapeutic decisions on their detection, despite a lack of data to establish clinical benefit. 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 beta-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 associated with a highly suggestive scarlatina-form rash in some patients. *Neisseria gonorrhoeae* and *Corynebacterium diphtheriae*, in specific patients and epidemiologic settings, may also cause pharyngitis [193]. *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* may also cause pharyngitis, but are rarely tested for in that context alone.

Respiratory viruses are the most common cause of pharyngitis in both adult and pediatric populations; however, it is unnecessary to define a specific etiology in patients with pharyngitis due to respiratory viruses since there exists no pathogen-directed therapy for these agents. 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 three agents [193].

Studies have shown a relationship between *Fusobacterium necrophorum* and pharyngitis in some patients, especially those with chronic sore throat. In this case, throat infection could be a prelude to Lemierre's syndrome though a direct association has not been demonstrated. *F. necrophorum* is an anaerobic organism and as such, requires additional media and the use of anaerobic isolation and identification procedures, which most laboratories do not perform from throat specimens. Molecular methods are not widely available outside of research settings. There is no data to support the use of specific diagnostics for *Fusobacterium* in acute pharyngitis [199-202].

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Although not (necessarily) associated with symptomatic pharyngitis, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* may reside in and be transmitted to and from the pharynx. Screening is recommended in populations at-risk [203, 204]. Nucleic acid amplification tests are recommended for this purpose, and some are now FDA-approved for testing of pharyngeal samples. While self-collected samples are recommended for this screening, NAATs on self-collected pharyngeal samples are currently not FDA-cleared and require in-house validation. Providers need to confirm with the laboratory if these specimens will be tested [205].

Table 22. Laboratory Diagnosis of Otitis Media

Etiological Agents[1]	Diagnostic Procedures	Optimum Specimens	Transport Issues
Moraxella catarrhalis *Haemophilus influenzae (non-typeable)* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Pseudomonas aeruginosa* *Alloiococcus otitidis* *Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae*	Gram stain, Aerobic bacterial culture	Tympanocentesis fluid	Sterile container, RT, ≤2 h
		Mini-tipped swab of fluid draining from the middle ear cavity in patients with myringotomy tubes or otorrhoea	Swab transport device, RT, ≤2 h

[1]Viruses are often etiologic agents but microbiologic studies do not assist treatment decisions

Table 23. Laboratory Diagnosis of Sinusitis

Etiological Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Acute Maxillary Sinusitis			
Bacterial			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Staphylococcus aureus*[1] *Streptococcus pyogenes*[1]	Gram stain Aerobic bacterial culture	Aspirate obtained by antral puncture	Sinus secretion collector (vacuum aspirator) Sterile container, RT, <2h
		Middle meatal swab specimen obtained with endoscopic guidance	Swab transport device, RT, <2 h
Complicated Sinusitis			
Bacterial			
Streptococcus pneumoniae *Haemophilus influenzae* *Moraxella catarrhalis* *Staphylococcus aureus* *Streptococcus pyogenes* *Pseudomonas aeruginosa* Enterobacterales	Gram stain Aerobic and anaerobic bacterial culture	Aspirate obtained by antral puncture[2]	Sinus secretion collector (vacuum aspirator) Sterile anaerobic

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Mixed aerobic-anaerobic microbiota from the oral cavity			container, RT, <2h[3]
		Tissue or aspirate obtained surgically	Sterile anaerobic container, RT, <2h
Fungal			
Aspergillus spp Zygomycetes *Fusarium* spp Other molds	Calcofluor-KOH stain Fungus culture	Aspirate obtained by antral puncture[2]	Sinus secretion collector (vacuum aspirator) Sterile aerobic container, RT, <2h
		Tissue or aspirate obtained surgically	Sterile aerobic container, RT, <2h

[1]*Staphylococcus aureus* and *Streptococcus pyogenes* do cause acute maxillary sinusitis but only infrequently [67].
[2]Antral puncture is a useful method for sampling the maxillary sinuses.
[3]Anaerobic transport vials are good for both aerobic and anaerobic bacteria.

Table 24. Laboratory Diagnosis of Pharyngitis

Etiological Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacterial			
Streptococcus pyogenes	Rapid direct antigen test (followed by a secondary test if negative)[1]	Dual pharyngeal swab	Swab transport device, RT, <2 h
	Direct nucleic acid amplification test (NAAT)[2] Nucleic acid probe tests[2]	Pharyngeal swab Pharyngeal swab	Swab transport device, RT, stability as specified by lab / manufacturer. Specific swabs/transport media may be required for each different NAAT in some cases.
Groups C and G beta-hemolytic streptococci[3] *(S. dysgalactiae, S. canis, or S. equi)*	Throat culture and antigen tests on isolates for Groups C and G streptococci	Pharyngeal swab	Swab transport device, RT, <2 h
Arcanobacterium haemolyticum[4]	Throat culture for *A. haemolyticum*	Pharyngeal swab	Swab transport device, RT, <2 h
Neisseria gonorrhoeae[4]	Throat culture for *N. gonorrhoeae*	Pharyngeal swab	Swab transport device, RT, <2 h

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	Direct nucleic acid amplification test (NAAT)	Pharyngeal swab	Swab transport device, RT, stability as specified by lab / manufacturer. Specific swabs/transport media may be required for each different NAAT in some cases.
Corynebacterium diphtheriae[4]	Methylene blue stain *C. diphtheriae* culture	Pseudomembrane	Sterile container, RT, <2h
Fusobacterium necrophorum	Anaerobic incubation. A selective medium is available	Pharyngeal swab	Anaerobic swab transport, RT, <2 h
Viral			
Epstein-Barr virus (EBV)	Monospot test[5] EBV serology	5 ml serum	Clot tube, RT, <2 h or refrigerated <24h
Herpes Simplex virus (HSV) [usually Type 1]	Direct detection test (DFA/NAAT) or Culture[6]	Swab of pharyngeal lesion	Swab transport device, RT, <2 h
Human immunodeficiency virus (HIV)	(see XIV Viral Syndrome)		
Screening for STI[8]			
Neisseria gonorrhoeae and *Chlamydia trachomatis*	Direct nucleic acid amplification test (NAAT)	Pharyngeal swab	Swab transport device, RT, stability as specified by lab / manufacturer. Specific swabs/transport media may be required for each different NAAT in some cases.

[1]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 [195]. This secondary testing is not necessarily required in adults [196]. 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.

[2]Direct and amplified NAATs for *Streptococcus pyogenes* are more sensitive than direct antigen tests and, as a result, negative direct NAAT results do not have to be arbitrated by a secondary test. The swab transport device should be

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compatible with the NAAT used. Direct nucleic acid probe tests are usually performed on enriched broth cultures, thus requiring longer turnaround times; some amplified tests for point of care use are quite rapid.
[3]Detection of Groups C and G beta-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.
[4]*Arcanobacterium haemolyticum, Neisseria gonorrhoeae* and *Corynebacterium diphtheria* cause pharyngitis only in limited epidemiologic settings. Few laboratories will routinely recover these organisms from throat swab specimens. If a clinical suspicion exists for one of these pathogens, the laboratory should be notified so that appropriate measures can be applied.
[5]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 d later at which time, if the patient had EBV infection, the Monospot is more likely to be positive. See section on viral diagnosis.
[6]Probable cause of pharyngitis only in immunocompromised patients. Numerous rapid tests based on detecting HSV directly (by DFA or NAAT) directly in clinical material have been developed; however the non-specific 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 which is compatible with the test to be performed.
[7]HSV serology (IgM and IgG) is not recommended for diagnosis of pharyngitis.
[8]Consult current guidelines for indications for pharyngeal screening in specific populations.

VIII. 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. The most recent of these is SARS CoV-2, which is responsible for the current unprecedented global pandemic which began in 2019. This section describes the major etiologic agents and the microbiologic approaches to the diagnosis of bronchitis and bronchiolitis; community-acquired pneumonia (CAP); hospital-acquired (HAP) and ventilator-associated pneumonia (VAP); infections of the pleural space; bronchopulmonary infections in patients with cystic fibrosis; and pneumonia in the immunocompromised host. The reader is referred to various practice guidelines that have been written in recent years by the Infectious Diseases Society of America, the American Thoracic Society, the American Academy of Pediatrics, and the American Society for Microbiology among other clinical practice groups that describe the clinical features, diagnostic approaches and patient management aspects of many of these syndromes.

Below is a summary of some important best practices and caveats when obtaining specimens for the diagnosis of respiratory infections.

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

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- NAATs have largely replaced rapid antigen tests and culture for respiratory virus detection.
- Providers should consult the laboratory's test directory for specific instructions prior to collection of specimens for fastidious pathogens (e.g., *Bordetella pertussis).*
- When collecting sputum for bacterial culture, obtaining first morning expectorated sputum is recommended. Blood cultures that accompany sputum specimens may occasionally be helpful, particularly in high-risk patients with CAP and patients at high risk for MRSA or *Pseudomonas aeruginosa*.
- Multiplex molecular pneumonia syndromic panels, when used judiciously, may provide earlier opportunity for therapeutic optimization than traditional cultures.
- The range of pathogens causing exacerbations of lung disease in cystic fibrosis patients has expanded to include fungi and mycobacteria. Specimens for mycobacterial and fungal cultures should be collected in patients with advanced disease.
- In the immunocompromised host, a broad diagnostic approach based upon invasively obtained specimens is suggested.
- Bronchoscopy with washings is the optimal diagnostic specimen in pediatrics with severe CAP when other diagnostic tests are not revealing.

Bronchitis and Bronchiolitis

Table 25 lists the etiologic agents and diagnostic approaches for bronchiolitis, acute bronchitis, acute exacerbation of chronic bronchitis and pertussis, clinical syndromes that involve inflammation of the tracheobronchial tree [206, 207]. Bronchiolitis, characterized by bronchospasm (wheezing) resulting from acute inflammation, airway edema, and increased mucous production, is the most common lower respiratory tract infection in children [206, 207]. Respiratory syncytial virus (RSV), human rhinovirus and human bocavirus type 1 are the major etiologies along with the less frequent list of other viruses listed in Table 25 [207]. SARS CoV-2 is an infrequent cause of bronchiolitis. Viral coinfections are not uncommon and have been observed in up to 30% of cases [207].

Acute bronchitis is characterized by inflammation in large airways resulting in cough with or without sputum production. Acute bronchitis is largely caused by viral pathogens, most commonly influenza A and B, rhinovirus, coronaviruses, parainfluenza viruses, human metapneumovirus and RSV. In 10% of cases, bacterial etiologies such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Bordetella pertussis* are seen [208]. *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. Consider pertussis in an adolescent or young adult with prominent paroxysmal or prolonged cough.

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Several FDA-approved NAAT platforms are available for the detection of a broad range of respiratory viruses and some of the "atypical bacteria" such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and in some cases *B. pertussis* associated with respiratory syndromes (see Table 26) [209, 210]. These have largely replaced rapid antigen detection tests and culture in most institutions. Performance characteristics vary among the various syndromic respiratory pathogen panels. Specimen sources may also vary depending upon the assay. Readers should become familiar with the platforms offered in their respective institutions and the approved specimen sources, collection devices and transport requirements. If *B. pertussis* is the major consideration based upon the clinical presentation, then a targeted NAAT test is the best option. Currently there are several FDA cleared assays specifically for *B. pertussis* detection. Literature suggests that detection of *B. pertussis* using multiplexed syndromic respiratory pathogen panel tests may be suboptimal compared to targeted singleplex NAATs [211, 212]. A targeted NAAT in combination with culture are the optimal tests of choice for *B. pertussis* detection. The Centers for Disease Control and Prevention have suggested best practices when using molecular tests for pertussis detection (https://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html).

Community-acquired pneumonia

The diagnosis of CAP is based on the presence of specific symptoms and suggestive radiographic features, such as pulmonary infiltrates and/or pleural effusion. Carefully obtained microbiological data can support the diagnosis, but often fails to provide an etiologic agent. Table 27 lists the more common causes of CAP and tests for diagnosis. A recent systematic review showed that *S. pneumoniae* is still the most common bacterial cause of CAP followed by *Haemophilus influenzae*, *Staphylococcus aureus* and *Enterobacterales* [213]. The "atypical" bacterial pathogens, *M. pneumoniae*, *C. pneumoniae*, and *Legionella pneumophila* account for 3-11% of cases [213]. Other less common etiologies may need to be considered depending upon recent travel history or exposure to vectors or animals that transmit zoonotic pathogens such as Sin Nombre virus (hantavirus pulmonary syndrome) or *Yersinia pestis* (pneumonic plague, endemic in the western United States). Due to availability of broad-based molecular viral respiratory pathogen tests and pneumonia syndromic panels that include viruses, respiratory viruses in some pre-COVID series accounted for as many as 30% of cases [213]. In many of the latter cases, bacterial coinfection was reported in up to 40% of cases [213, 214].

Newer syndromic panel tests for the detection of respiratory pathogens are listed in Table 26. The rationale for attempting to establish an etiology is that identification of a pathogen will focus the antibiotic management for a particular patient [215]. In addition, identification of certain pathogens such as *Legionella* species, influenza viruses, and SARS CoV-2 have important public health significance. Current IDSA/ATS practice guidelines consider diagnostic testing as optional for the patient who is not hospitalized or who is hospitalized with mild CAP [216, 217]. In those patients who require admission for severe CAP or who have strong risk factors for MRSA or *Pseudomonas aeruginosa*, blood culture sets should be collected before initiating antimicrobial

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therapy. Culture and Gram stain of good quality samples of expectorated sputum, when available, should be obtained for patients requiring hospitalization. Urinary antigen tests for *S. pneumoniae* and *L. pneumophila,* where available, should be considered for patients with severe CAP. In patients with non-severe disease and strong epidemiological risk factors for *Legionella* infection, testing for *Legionella* is warranted [216, 218]. In geographic areas where *Legionella* species other than *L. pneumophila* are more prevalent, culture for *Legionella* should be performed if clinically indicated. The recommendations for children agree with the adult recommendations with respect to when to obtain blood cultures and sputum cultures but differ slightly for other laboratory tests [219]. For example, *S. pneumoniae* urinary antigen testing is not recommended because false positive tests are common [219].

Testing for influenza viruses in both outpatient and inpatient settings during periods of community spread is a strong recommendation in the 2021 ATS clinical practice guideline [216, 218, 220]. Testing recommendations for non-influenza viral pathogens (released before the SARS CoV-2 pandemic; see section XVI) endorse the use of NAATs in hospitalized patients with severe CAP or in patients with immunocompromising conditions based upon the reported findings of high inpatient mortality associated with non-influenza viruses in these vulnerable populations [218-221]. Although a weak recommendation, in children with appropriate signs and symptoms, *M. pneumoniae* testing is indicated. There are several molecular assays available for *M. pneumoniae* detection and *C. pneumoniae* detection [209, 210]. The molecular syndromic pneumonia panel tests also detect *Legionella pneumophila* (Table 26) [222].

Laboratories must have a mechanism in place for screening sputum samples for acceptability (to exclude those that are heavily contaminated with oropharyngeal microbiota and not representative of deeply expectorated samples) prior to setting up routine bacterial culture. Poor quality specimens provide misleading results and should be rejected because interpretation would be compromised. Endotracheal aspirates or bronchoscopically obtained samples (including "mini BAL" using the Combicath [KOL Bio Medical Instruments, Chantilly, 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.

Mycobacterial infections should be in the differential diagnosis of CAP that fails to respond to therapy for the typical CAP pathogens. *Mycobacterium tuberculosis,* is an important pathogen among persons from areas of high endemicity. According to the World Health Organization, tuberculosis saw a global resurgence during the SARS CoV-2 pandemic [Global Tuberculosis Report 2022 (who.int)]. *Mycobacterium avium* complex and other non-tuberculous mycobacteria, such as *M. abscessus*, *M. kansasii*, and *M. xenopi*, are also important, not just among patients with HIV, but especially in patients with chronic lung disease or cystic fibrosis and in middle-aged or elderly thin women [223, 224].

Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia

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Hospital-acquired and ventilator-associated pneumonias (HAP and VAP, respectively) are frequently caused by *S. aureus* and Gram-negative pathogens, with up to 34% or the latter being multi-drug resistant bacteria [225, 226]**.** Aside from respiratory viruses that may be nosocomially transmitted, viruses and fungi are rare causes of HAP and VAP in the immunocompetent patient. Patients admitted with SARS CoV-2 experience higher rates of HAP and VAP compared to patients with influenza or patients with no viral infection [226]. Table 28 lists the organisms most commonly associated with pneumonia in the immunocompetent patient with HAP or VAP.

The 2016 IDSA and ATS guidelines recommend noninvasive sampling of the respiratory tract for both HAP and VAP [227]. In the non-ventilated patient, the specimens could include those obtained by spontaneous expectoration, sputum induction or nasotracheal suction in an uncooperative patient and, in the ventilated patient, endonasotracheal aspirates are preferred [227]. Determining the cause of the pneumonia relies upon initial Gram stain and semi-quantitative cultures of endotracheal aspirates or sputum. A smear lacking inflammatory cells and a culture absent of potential pathogens have a very high negative predictive value. Cultures of endotracheal aspirates, while likely to contain the true pathogen, also consistently grow more mixtures of species of bacteria than specimens obtained by bronchoscopic techniques. This may lead to additional unnecessary antibiotic therapy. Quantitative assessment of invasively obtained samples such as BAL fluid and protected specimen brush specimens is often performed [227]. 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 colony-forming units (CFU)/mL; BAL, 10^4 CFU/mL; protected specimen brush samples (PSB), 10^3 CFU/mL [226]. Quantitative studies require extensive laboratory work and special procedures that laboratories may not accommodate. Therefore, quantitative cultures are not endorsed by the guidelines despite studies that show decreased antibiotic utilization with their use [227]. Bronchial washes are not appropriate for routine bacterial culture.

Two molecular pneumonia syndromic panel tests are available for the evaluation of patients with HAP and VAP, the FilmArray Pneumonia panel (BioFire, Inc., Salt Lake City, UT) and Unyvero HPN (OpGen, Inc. Rockville, MD **(**Table 26**).** Data on potential clinical impact are largely derived from retrospective studies [222, 225, 228, 229]. These studies show early de-escalation of antimicrobial agents in 39-48% of patients and escalation in 21-22% [222, 225, 228, 229]**.** At the time of writing, several randomized controlled trials are in progress to assess the clinical impact of these pneumonia panels on patients with HAP/VAP [225]. Laboratories that implement pneumonia syndromic panel testing may wish to work with antimicrobial stewardship and other key stakeholders to ensure appropriate utilization and interpretation of test results. For example, these tests are most useful in the hospitalized patient at risk for *S. aureus* or *P. aeruginosa* who is not receiving antimicrobial therapy and who has clear documentation of a change in respiratory status and radiographic evidence of a new infiltrate

Infections of the Pleural Space

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An aging population among other factors has resulted in an increase in the incidence of pleural infection [230]. Pneumonia is the most common predisposing factor in pleural space infection and as many as 60% of patients with pneumonia develop some type of effusion [231]. While the majority are simple effusions, up to 30% represent complicated parapneumonic effusions or frank empyema [231]. The infectious causes of pleural effusions differ between community-acquired and hospital-acquired disease [232, 233]. In a large multicenter study (MIST1) of 454 adult patients with pleural infection to assess streptokinase treatment, the major pathogens recovered in decreasing order of frequency were *Streptococcus anginosus* group, *S. aureus*, anaerobic bacteria, other streptococci, *Enterobacterales*, and *S. pneumoniae* [232]. Among patients with hospital-acquired infection, *S. aureus* tops the list, with 33%-50% being methicillin-resistant, followed by aerobic Gram-negatives, the streptococci (*S. anginosus* group, *S. pneumoniae*), *Enterococcus* spp and anaerobes [232-234]. Table 29 summarizes the major pathogens. Any significant accumulation of fluid in the pleural space should be sampled by thoracentesis. Specimens should be delivered immediately to the laboratory or placed into appropriate anaerobic media for transport. In some institutions, inoculation into blood culture bottles has become an established practice. This is acceptable and has been shown to increase the sensitivity by 20% [233, 234]. The manufacturer's guidelines should be followed with respect to the volume inoculated and other considerations. If blood culture bottles are used, an additional sample should be sent to the microbiology laboratory for Gram stain and culture particularly if non-bacterial pathogens are suspected. Even when optimum handling occurs, cultures may fail to yield an organism in up to 50% of cases [233]. Laboratory developed NAATs targeting pneumococcal genes, such as those that encode pneumolysin and autolysin, in fluid from pediatric cases of pleural infection, have been very useful in establishing an etiology [230].

Fluid should be sent for cell count and differential, pH, total protein, glucose, lactate dehydrogenase (LDH) and cholesterol. These values assist with the determination of a transudative or exudative process and in the subsequent management of the syndrome. A meta-analysis showed that the best predictors of an exudate were pleural fluid cholesterol level > 55 mg/dL and an LDH greater than 200 U/L or the ratio of pleural fluid cholesterol to serum cholesterol greater than 0.3 [235]. Infections result in an exudate or polymorphonuclear leukocytes (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, molecular testing, and histopathology increases the diagnostic sensitivity. The IDSA/ ATS/CDC guidelines on the diagnosis of tuberculosis in adults and children recommend the measurement of adenosine deaminase (ADA) and free interferon-λ (IFN-λ) in pleural fluid. This endorsement is based upon a sensitivity and specificity of ADA of $\geq$ 79% and $\geq$ 83%, respectively as determined by several meta-analyses [235]. The figures for free IFN-λ were $\geq$ 89% and $\geq$ 97 % for sensitivity and specificity, respectively [236]. It should be stressed that the quality of evidence is low and both markers should be used in conjunction with hematologic and chemical parameters and other diagnostic tests such as NAAT, culture, and histology of a pleural biopsy. The performance of ADA in developed countries has been shown to be quite variable and is related to multiple factors including

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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 [237].

Pulmonary Infections in Cystic Fibrosis

Patients with cystic fibrosis suffer from chronic lung infections due to disruption of exocrine function that does not allow them to clear microorganisms that enter the distal airways of the lung. The spectrum of organisms associated with disease continues to expand and studies of the microbiome demonstrate the complex interactions between easily cultivatable and non-cultivatable organisms. Table 30 lists the most frequently isolated pathogens in this patient population. Early in childhood, organisms frequently seen in pediatric patients without cystic fibrosis, such as *S. pneumoniae*, *H. influenzae*, and *S. aureus*, cause infections. Of these organisms, methicillin-resistant *Staphylococcus aureus* (MRSA) has significantly increased in prevalence [238]. During adolescence to early adulthood, *P. aeruginosa* becomes the most important pathogen involved in chronic lung infection and the concomitant lung destruction that follows, for many patients with cystic fibrosis. 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, Achromobacter xylosoxidans* and *Achromobacter ruhlandii* may be acquired during a hospital or clinic visit [238]. *Burkholderia cepacia* complex is a very important pathogen in these patients. *B. cenocepacia* is highly pathogenic and is responsible for rapid decline and death in a subset of patients who acquire the virulent clones. Other *B. cepacia* complex species that contribute to cystic fibrosis lung disease include *B. multivorans*, *B. dolosa* and *B. vietnamiensis* [239]. 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 cystic fibrosis lung disease is still unclear, include *B. gladioli, Ralstonia* spp, *Cupriavidus* spp, *Inquilinus* spp, *Herbaspirillum* spp, *Chryseobaterium* spp, *Sphingobacterium* spp and *Pandorea* [238-240]. The reader is referred to the Parkins reference for a discussion of pathogens within the cystic fibrosis microbiota [238].

As cystic fibrosis patients have survived into adulthood, opportunistic pathogens such as non-tuberculous mycobacteria (NTM) have been isolated with increasing frequency ranging in prevalence from 6% to up to 30% in patients aged > 40 years [238]. The *M. avium* complex and the *Mycobacterium abscessus* complex are the most commonly encountered NTM [238]. There is evidence to suggest that both *M. abscessus* and *M. avium* complex contribute to lung destruction and should be treated when cultures are repeatedly positive. Mycobacterial culture should be added to the routine cultures obtained from patients >15 years of age who present with exacerbations, as the incidence of *Mycobacterium* species is likely underestimated due to failure to routinely assess patients for these organisms [240]. Note that specimens from patients with cystic fibrosis for mycobacterial culture require additional decontamination steps to prevent *P. aeruginosa* contamination.

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Aspergillus fumigatus is the most common fungus recovered from patients with cystic fibrosis, in whom it causes primarily allergic bronchopulmonary disease. *Scedosporium apiospermum* complex, *Lomentospora prolificans*, *Apiotrichum* (*Trichosporon*) *mycotoxinivorans, Exophiala dermatitidis* and *Rasamsonia agrillacea* complex may cause chronic colonization of the cystic fibrosis airway and contribute to disease depending upon individual patient factors [241]. Table 30 outlines current laboratory approaches to the recovery and detection of pathogens important in cystic fibrosis lung disease exacerbations. Laboratories should spend resources on those pathogens proven or likely to play a significant role in pulmonary decline in these patients.

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 [242].

Virtually any potential pathogen may result in significant illness and the challenge for both clinicians and microbiologists is to differentiate infectious from non-infectious causes of pulmonary infiltrates. Factors to consider include the type of immunosuppression, solid organ vs human stem cell transplantation, and the radiographic appearance of the pulmonary process [242-244]. In addition, the likelihood of a specific infection may be affected by epidemiological considerations and recently administered prophylaxis. Table 31 focuses on the major infectious etiologies likely to be of interest in most immunocompromised hosts [242-244]. Patients are still vulnerable to the usual bacterial and viral causes of CAP and HAP. In addition, fungi, herpesviruses and even parasites such as *Toxoplasma gondii* and *Strongyloides stercoralis* may play a more significant role and should be considered depending upon the immune defect [242-247].

More definitive procedures to sample the lung are required when rapid and noninvasive tests, such as urine or serum antigen tests and rapid viral diagnostics, are not revealing. Several diagnostic procedures can be performed, but usually the patient initially undergoes bronchoscopy with BAL with or without transbronchial biopsy. When an infiltrate is focal, it is important to wedge the scope in the pulmonary segment corresponding to the abnormality on radiographs; otherwise, in diffuse disease, the scope is usually wedged in the right middle-lobe or lingula. It is suggested that microbiology laboratories in collaboration with infectious diseases physicians and pulmonologists, develop an algorithm for processing samples that includes testing for all major categories of pathogens as summarized in the table. Cytologic analysis and/or histopathology may assist with interpretation of positive NAAT 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 culture and, when available, NAAT [242, 244, 247]. Although frequently over-utilized in non-immunocompromised patients, serum and BAL galactomannan and serum 1,3 b-D-glucan tests are helpful in patients in whom

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radiographic or clinical evidence suggests fungal pneumonia[248, 249]. However, cytology and/or histopathology are quite useful for distinguishing conditions such as pulmonary hemorrhage and rejection from infectious causes of infiltrates. Transthoracic needle aspiration, computed tomography-guided biopsies of pleural-based lesions and open lung biopsies likewise may be considered if less invasive diagnostics are unrevealing.

Currently, progress is being made with metagenomic next generation sequencing methods. Serum cell free DNA sequencing (Karius test, Redwood, CA) for testing patients in whom standard of care methods are unrevealing has been used to diagnose fungal pneumonia in hematopoietic cell transplant recipients with some success [250]. The disadvantage of this assay is the expense and the fact that samples must be sent to Karius for testing. Other published mNGS applications for the diagnosis lower respiratory tract infections are slow, labor-intensive and expensive; interpretation of sequencing results may be challenging [251]. However, these technologies have the advantage of detecting pathogens that are not easily be cultivatable, were partially treated or otherwise not considered in the differential diagnosis [251]. This is a rapidly evolving area and the reader is referred to current literature for additional information on this topic.

Table 25. Laboratory Diagnosis of Bronchiolitis, Bronchitis, and Pertussis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bronchiolitis			
Viruses[a]			
Respiratory syncytial virus Rhinovirus Human bocavirus type I Human Metapneumovirus Parainfluenza virus Adenovirus Human Coronaviruses (HCoV NL63; HKCoV-2) Adenovirus Influenza virus Enterovirus	NAAT[b]	NP swabs, Nasal aspirates or nasal washes preferred; , throat washes or swabs	VTM, UTM or sterile container (washes, etc.) transport RT, < 2 h or refrigerated (2-8□C), 24-48
	Rapid antigen detection tests[c] Virus culture[d]	NP swabs or aspirates, nasal washes preferred NP swabs, NP aspirates, nasal washes, throat washes or swabs	VTM or sterile container (washes, etc.) transport RT < 2h or refrigerated (2-8□ C, 24 h-48 h)
Acute Bronchitis			
Bacteria			
Mycoplasma pneumoniae	NAAT[e]	Throat swab[e], nasopharyngeal (NP) swab, NP aspirates	NP swab, aspirate or wash Suitable transport device, RT, 2h
	Mycoplasma IgG and IgM serology (enzyme immunoassay [EIA])	5 mL serum	Clot tube, RT, 2 h

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Chlamydia pneumoniae	NAAT[e]	NP swab	Suitable transport device, RT, 2h
	Chlamydia IgG and IgM serology MIF)	5 mL serum	Clot tube, RT, 2 h
Bordetella pertussis	NAAT *Bordetella* culture on Regan Lowe or Bordet-Gengou selective agar and NAAT	Flocked swabs or swabs tipped with polyester (Dacron®), rayon, nylon[g] NP swabs (preferred); NP aspirates (preferred); nasal wash	RT, 2h Use suitable transport[h], RT, ≤ 24 h
Viruses			
Influenza viruses Parainfluenza viruses Respiratory syncytial virus Human metapneumovirus Coronavirus Adenovirus Rhinovirus	NAAT[b]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	VTM or sterile container (washes, etc. transport RT < 2 h or refrigerated (2°-8□ C), 24-48 h
	Rapid antigen detection tests[c] Virus culture[d]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	VTM or sterile container (washes, etc. transport RT < 2 h or refrigerated (2°-8□C), 24-48 h
Acute Exacerbation of Chronic Bronchitis			
Bacteria			
Haemophilus influenzae (non-typeable)	Gram stain Aerobic bacterial culture	Expectorated sputum	Sterile container, RT, 2 h or > 2-24 h, 2-8□ C
Moraxella catarrhalis			
Chlamydia pneumoniae	See above under Acute bronchitis	See *Chlamydia* and *Mycoplasma* above	See above
Mycoplasma pneumoniae	See above under Acute bronchitis	See *Chlamydia* and *Mycoplasma* above	See above
Streptococcus pneumoniae	Gram stain Aerobic bacterial culture		
	Urine antigen[f]	First voided clean catch urine specimen	Sterile container, RT, 2 h
Pseudomonas aeruginosa	Gram stain Aerobic bacterial culture	Expectorated sputum	Sterile container, RT, 2 h or > 2-24 h, 2°C-8° C
Viruses			
Rhinovirus Coronavirus Parainfluenza virus (most often PIV3) Influenza virus Respiratory syncytial virus	NAAT[b]	Nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs	VTM or sterile container (washes, etc. transport RT < 2 h or refrigerated (2°C-8□C), 24-48 h

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Human metapneumovirus Adenoviruses	Rapid antigen detection tests[c] Virus culture[d]		

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

[a] Viruses are listed in decreasing order of frequency [207].

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

[c] Rapid antigen tests for respiratory virus detection lack sensitivity, and depending upon the product, specificity. A meta-analysis of rapid influenza antigen tests showed a pooled sensitivity of 62.3% and a pooled specificity of 98.2% [252]. They should be considered as screening tests only. At a minimum, a negative result should be verified by another method. Specimen quality is critical to optimize these tests.

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

[e] There are several FDA cleared assays available at this time. At least five comprehensive multiplex panels contain *M pneumoniae* and *C pneumoniae* as part of a comprehensive respiratory syndromic panel test (see Table 26). There is one FDA-cleared singleplex assay for *M pneumoniae*. Availability is laboratory specific. Clinicians should check with the laboratory for validated specimen sources, collection and transport, performance characteristics and turnaround time. In general, avoid calcium alginate swabs and mini-tip swabs for nucleic acid amplification tests.

[f]Sensitivity in non-bacteremic patients with pneumococcal pneumonia is 52-78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80-86%; specificity in adults is > 90%. However, studies have reported a 21-54% false positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [242, 253].

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

[h] Plating of specimens at the bedside is ideal but rarely done. Several types of transport media are acceptable**.** These include casamino acid solution, Amies transport medium Regan-Lowe transport medium (Hardy Diagnostics, Inc.) [254].

TABLE 26: FDA-Cleared Broad Based Molecular Syndromic Panel Tests for Respiratory Pathogen Detection*

Assay	Manufacturer	Viral Pathogens	Bacterial Pathogens/Resistance Markers	Specimen	TAT
BioFire® FilmArray Respiratory Panel 2.1	BioFire Diagnostics, LLC, Salt Lake City, UT	Influenza A, A/H1, A/H3, A/H1-2009; Influenza B, RSV, Parainfluenza viruses 1-4, Human metapneumovirus, Human Rhinovirus/Enterovirus, Adenovirus, Coronavirus HKU1, NL63, 229E and OC43, SARS CoV-2	*Bordetella pertussis; Bordetella parapertussis Chlamydia pneumoniae Mycoplasma pneumoniae*	NP swab in transport media or saline	45 min
BioFire® FilmArray Respiratory Panel EZ 2.0 (CLIA	BioFire Diagnostics, LLC. Salt Lake City, UT	Influenza A, A/H1, A/H3, A/H1-2009; Influenza B, RSV, Parainfluenza virus, Human metapneumovirus, Human	*Bordetella pertussis Chlamydia pneumoniae Mycoplasma pneumoniae*	NP swab in transport media or saline	1 h

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Waived)[b]		Rhinovirus/Enterovirus, Adenovirus, Coronavirus			
Applied BioCode® Respiratory Pathogen Panel	Applied BioCode, Inc. Santa Fe Springs, CA	Influenza A, A/H1, A/H3, A/H1-2009; Influenza B, RSV, Parainfluenza viruses 1-4, Human metapneumovirus, Human Rhinovirus, Adenovirus, Coronavirus HKU1, NL63, 229E and OC43	*Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*	NP swab in viral transport media	4 h
GenMark ePlex Respiratory Pathogen Panels RP and RP2+	GenMark, Inc., Carlsbad CA	Influenza A, A/H1, A/H3, A/H1-2009; Influenza B, RSV A and B, Parainfluenza viruses 1-4, Human metapneumovirus A/B, Human Rhinovirus/Enterovirus, Adenovirus, Coronavirus , SARS CoV-2	*Chlamydia pneumoniae* *Mycoplasma pneumoniae*	NP swab in viral transport media BAL fluid in sterile container (RP assay only)	1.5 h
Luminex Verigene® Respiratory Pathogens *Flex* Test	Luminex Inc. Austin, TX	Influenza A, subtypes A/H1, A/H3 Influenza B, RSV A and B, Human rhinovirus, Parainfluenza viruses 1-4 Human metapneumovirus Adenovirus	*Bordetella parapertussis/B bronchiseptica* *Bordetella pertussis; Bordetella holmesii*	NP swab in viral transport media Sputum, BAL fluid in sterile container Endonasotracheal aspirates	2 h
Luminex NxTAG® Respiratory Pathogen Panel	Luminex, Inc. Austin TX	Influenza A, A/H1, A/H3, Influenza B, RSV A and B, Parainfluenza viruses 1-4, Human metapneumovirus, Human Rhinovirus/Enterovirus, Adenovirus, Coronavirus HKU1, NL63, 229E and OC43, Human Bocavirus	*Chlamydia pneumoniae* *Mycoplasma pneumoniae*	NP swab in viral transport media	5 h
Luminex NxTAG RVP FAST v2	Luminex	Influenza A, A/H1, A/H3, Influenza B, RSV, Parainfluenza viruses 1-4, Human metapneumovirus, Human Rhinovirus/Enterovirus, Adenovirus, Coronavirus HHU1, NL63, 229E and OC43	N/A	NP swab in viral transport media	3.5 h
QIAstat Respiratory Panel	Qiagen	Influenza A, A/H1, A/H3, A/H1-2009Influenza B, RSV A and B, Parainfluenza viruses 1-4, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Adenovirus, Coronavirus HHU1, NL63, 229E and OC43, SARS CoV-2[c]	*Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*	NP swab in universal transport media	

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Comprehensive Pneumonia Panels					
BioFire® FilmArray Pneumonia Panel	BioFire Diagnostics, LLC Salt Lake City, UT	Influenza A, Influenza B, Adenovirus, Coronavirus, Parainfluenza virus, RSV, Human Rhinovirus/Enterovirus, Human Metapneumovirus	**Semi-quantitative** *Acinetobacter calcoaceticus-baumannii* complex *Enterobacter cloacae* *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* group *Moraxella catarrhalis* *Proteus spp* *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes* **Qualitative** *Legionella pneumophila* *Mycoplasma pneumoniae* *Chlamydia pneumoniae* **Resistance markers** Methicillin resistance *mecA/mecC* and MREJ ESBL (CTX-M) Carbapenemases KPC NDM Oxa-48-like VIM IMP	BAL (including mini-BAL) Sputum Endotracheal aspirates	1 h
Curetis Unyvero Lower Respiratory Panel[a]	OpGen	N/A	*Acinetobacter* spp *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Stenotrophomonas maltophilia*	Induced or expectorated sputum, nasal aspirates or washes, NP swabs or aspirates, throat washes or swabs, bronchoscopic specimens	< 5 h

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			Streptococcus pneumoniae *Legionella pneumophila* *Mycoplasma pneumoniae* *Chlamydia pneumoniae* **Resistance markers** Penicillin resistance (TEM) Methicillin resistance *mecA/mecC* and MREJ ESBL (CTX-M) Carbapenemases KPC NDM OXA-23, 24, 48, 58 VIM		

Abbreviations: TAT, turnaround time; NP, nasopharyngeal; BAL, bronchoalveolar lavage; N/A, not available
*Modified from references 214 and 215.
[a] This panel also detects *Pneumocystis jirovecii*.
[b] The RP2.1 EZ panel with SARS CoV-2 has FDA- EUA.
[c]The QIAstat respiratory panel has EUA for the SARS CoV-2 portion of the assay.

Table 27. Laboratory Diagnosis of Community-acquired Pneumonia

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria			
Streptococcus pneumoniae	Gram stain Culture	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; > 2-24 h, 4°C
	Urine antigen[a]	Urine	Sterile container, RT, 24 h; > 24 h-14 d, 2-8°C
Staphylococcus aureus *Haemophilus influenzae* *Enterobacterales* *Pseudomonas aeruginosa*	Gram stain Culture	Sputum, bronchoscopic specimens	Sterile container, RT, 2 h; > 2-24 h, 4°C
Legionella species	Urine antigen *L. pneumophila* serogroup 1	Urine	Sterile container, RT, 24 h; > 24 h-14 d, 2-8°C
	Selective culture on BCYE	Induced sputum, bronchoscopic specimens	Sterile container, RT, 2 h; > 2-24 h, 4°C
	NAAT[b]	Induced sputum, endonasotracheal aspirates, bronchoscopic specimens	Sterile container, RT, 2 h; > 2-24 h, 4°C
Mycoplasma pneumoniae	NAAT[b]	Throat swab, NP swab, sputum, BAL	Transport in M4 media or other *Mycoplasma*-specific medium at RT or 4°C up to 48 h; ≥48 h, -70°C
	Serology IgM, IgG antibody detection	Serum	Clot tube, RT, 24 h; > 24 h, 4°C

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Chlamydia pneumoniae	NAAT[b]	NP swab, throat washings, sputum, bronchial specimens	Transport in M4 or other *Mycoplasma*-specific medium at RT or 4ºC up to 48 h;≥48 h,-70ºC
	Serology (MIF) IgM antibody titer; IgG on paired serum 2-3 weeks apart	Serum	Transport in M4 or other specialized medium at RT or 4ºC up to 48 h;≥48 h,-70ºC Clot tube, RT, 24 h; >24 h, 4ºC
Mixed anaerobic bacteria (aspiration pneumonia)	Gram stain Aerobic and anaerobic culture	Bronchoscopy with protected specimen brush	Sterile tube with 1 mL of saline or thioglycolate; RT, 2 h; >2-24 h
		Pleural fluid (if available)	Sterile container RT, without transport media ≤ 60 min; Anaerobic transport vial RT, 72 h
Mycobacteria			
Mycobacterium tuberculosis and NTM	AFB smear AFB culture NAAT[d]	Expectorated sputum minimum 5 mL; induced sputum; bronchoscopically obtained specimens Gastric aspirates in pediatrics	Sterile container, RT,≤2 h; ≤24 h, 4ºC
Fungi			
Histoplasma capsulatum	Calcofluor - KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, <2 h; ≤24 h, 4ºC
	Histology	Tissue	Sterile container 4º C; formalin container, RT, 2-14 d
	Histoplasma antigen Tests	Serum, urine, pleural fluid (if available)	Clot tube, RT, 2 d; 2-14 d, 4ºC Sterile container (urine), RT 2 h; >2 – 72 h, 4ºC
	Histoplasma serum antibody (CF)	Serum	Clot tube, RT, 24 h; 4ºC, >24 h
Coccidioides immitis/posadasii	Calcofluor - KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens	Sterile container, RT, < 2 h; ≤24 h, 4ºC
	Histology	Tissue	Formalin container, RT, 2-14 d; Sterile container 2-14 d, 4ºC
	Coccidioides serum antibody IgM (ID, LA, EIA) IgG antibody (CF, EIA)	Serum	Clot tube, RT, 24 h;>24 h, 4ºC
Blastomyces dermatitidis	Calcofluor -KOH or other fungal stain Fungal culture	Expectorated sputum; induced sputum, bronchoscopically obtained specimens; tissue	Sterile container, RT, < 2h; ≤ 24h, 4ºC
	Histology	Tissue	Sterile container 4º C, formalin container, RT, 2-14 d
		Serum,	Clot tube, RT, 24 h

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	Blastomyces antigen Tests	Urine, BAL fluid, pleural fluid (if available)	Sterile container 4°C, 2-14 d
	Blastomyces serum antibody (CF)	Serum	Clot tube, RT, 24h; >24 h, 4°C
Viruses			
Influenza viruses A, B	Rapid antigen detection DFA Viral culture methods NAAT[c]	Nasal aspirates, nasal washes, NP swabs, throat washes, throat swabs, bronchoscopically obtained samples Transport in viral transport media, RT <2 h; 5 d, 4°C; >5 d, -70°C	
Adenovirus	DFA Viral culture methods NAAT[c]		
Parainfluenza viruses 1-4	DFA Viral culture methods NAAT[c]		
Respiratory syncytial virus	Rapid antigen detection DFA Viral culture methods NAAT[c]		
Human metapneumovirus	DFA NAAT[c]		
Coronaviruses including SARS CoV-2	NAAT[3c]		
Rhinovirus	Viral culture methods NAAT[c]		
Enteroviruses	Viral culture methods NAAT[c]		
Parasites			
Paragonimus westermani	Direct microscopic examination of pleural fluid and sputum for characteristic ova	Pleural fluid Sputum	Sterile container, fresh samples 4° C, 60 min; preserved samples, RT, >60 min-30 d

Abbreviations: BCYE, buffered charcoal yeast extract; NAAT, nucleic acid amplification test; NP, nasopharyngeal; BAL, bronchoalveolar lavage; DFA, direct fluorescent antibody test; CF, complement fixation; ID, immunodiffusion; LA, latex agglutination; EIA, enzyme immunoassay; KOH, potassium hydroxide; NTM, non-tuberculous mycobacteria.

[a] Sensitivity in non-bacteremic patients with pneumococcal pneumonia is 52-78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80-86%; specificity in adults is > 90%. Studies have reported a 21-54% false positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [242, 253].

[b] There are several US Food and Drug Administration (FDA) - cleared assays available at this time. Several assays are multiplex panels that contain *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* as part of a comprehensive respiratory syndromic panel and two pneumonia panels designed for severe CAP or HAP/VAP include *Legionella pneumophila* the BioFire FilmArray (BioFire, Inc. Salt Lake City, UT and Unyvero HPN (OpGen, Inc Rockville, MD). There is one singleplex assay for *M pneumoniae*. Availability is laboratory specific. See Table 26. Clinicians should check with the laboratory for validated specimen sources, collection and transport, performance characteristics and turnaround time. In general, avoid calcium alginate swabs and mini-tip swabs for NAATs. In geographic areas where *Legionella* species other than *L. pneumophila* are more prevalent, culture for *Legionella* should be performed if clinically indicated

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[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. See Table 26.
[d] Sensitivity of NAAT when AFB smear microscopy is positive is 96% and specificity is 85%; with AFB smear negative specimens, sensitivity is 66% and specificity is 98% [236].

Table 28. Laboratory Diagnosis of Hospital-Acquired Pneumonia and Ventilator-Associated Pneumonia (Immunocompetent Host)

Etiologic Agents	Diagnostic Procedures	Optimum specimens	Transport Issues
Bacteria			
Staphylococcus aureus and MRSA *Pseudomonas aeruginosa* *Klebsiella spp* *Escherichia coli* *Acinetobacter* spp *Enterobacter* spp *Serratia marcescens* *Stenotrophomonas maltophilia* *Haemophilus influenzae*	Blood culture Gram stain Quantitative or semi-quantitative aerobic and anaerobic culture[a]	Blood cultures Sputum Endotracheal aspirates BAL Protected specimen brush samples[a] Lung tissue	Routine blood culture bottles, RT ≤ 24 h Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
Streptococcus pneumoniae	As above plus urine antigen[b]	Urine	Sterile container RT, 24 h; >24 h-14 d, 2-8ºC
Mixed anaerobes (aspiration)	Gram stain Culture[a]	Protected specimen brush samples Lung tissue	Sterile tube with 1 mL of thioglycolate (for brush samples); Sterile container for tissue; RT, 2 h; 4ºC, >2-24 h
Legionella spp	Culture on BCYE media NAAT[c]	Induced sputum Endotracheal aspirates BAL Protected specimen brush samples Lung tissue	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
	Urine antigen (*L. pneumophila* serogroup 1 only)	Urine	Sterile container RT, <24 h; 4ºC >24 h -14 d
Fungi			
Aspergillus spp	Fungal stain—KOH with calcofluor; other fungal stains Fungal culture	Endotracheal aspirates BAL Protected specimen brush samples	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
	Histology	Lung tissue	Sterile cup; RT, 2 h; or formalin container, RT, 2-14 d
	Galactomannan[d] (1-3) □-D-glucan	Serum,	Clot tube 4ºC, ≤5 d; >5 d, -70ºC
		BAL	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
Viruses			

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Influenza viruses A, B Parainfluenza viruses 1-4 Adenovirus RSV	NAAT[e] Rapid antigen detection DFA Viral culture methods	Nasal washes, aspirates NP swabs, Endotracheal aspirates BAL Protected specimen brush samples	Transport in viral transport media, RT or 4°C, 5 d; -70° C, >5 d

Abbreviations: BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; NAAT, nucleic acid amplification test; NP, nasopharyngeal; DFA, direct fluorescent antibody.

[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 non-bacteremic patients with pneumococcal pneumonia is 52-78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80-86%; specificity in adults is > 90%. However, studies have reported a 21-54% false positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [242, 253].

[c]Two US Food and Drug Administration (FDA)-cleared molecular multiplex pneumonia panel tests, BioFire FilmArray pneumonia panel test (BioFire, LLC. Salt Lake City, UT and Unyvero HPN (OpGen, Inc Rockville, MD) include *Legionella pneumophila* on their panels. See Table 26. Availability is laboratory specific. Provider needs to check with the laboratory for optimal specimen source, performance characteristics and turnaround time. In geographic areas where *Legionella* species other than *L. pneumophila* are more prevalent, culture for *Legionella* should be performed if clinically indicated

[d]Performance characteristics of these tests are reviewed in references [245, 249].

[e]Several FDA-cleared respiratory viral NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Some have FDA-EUA for detection of SARs CoV-2. See Table 26. Readers should check with their laboratories regarding availability and performance characteristics including certain limitations.

Table 29: Laboratory Diagnosis of Infections of the Pleural Space

Etiologic Agents	Diagnostic Procedures	Optimum specimens	Transport Issues
Bacteria			
Aerobes			
Staphylococcus aureus	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2-24 h
Streptococcus pneumoniae	As above plus *S. pneumoniae* urinary antigen	Urine, Pleural fluid	Sterile container, RT, 24 h; >24 h-14 d, 2-8 °C; Sterile container, RT, 2 h; 4°C, >2-24 h
Streptococcus pyogenes *Streptococcus anginosus* group Other streptococci *Enterobacterales* *Enterococcus* spp *Pseudomonas aeruginosa*	Gram stain Culture	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2-24 h
Nocardia spp	Gram stain Modified acid fast stain Culture (include selective BCYE or other selective media)		
Legionella spp	Gram stain (note, *Legionella* spp. cells stain more intensely with	Pleural fluid	Sterile container, RT, 2 h; 4°C, >2-24 h

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	carbolfuchsin counter stain than with safranin) Culture on BCYE		
	Legionella urinary antigen (*L. pneumophila* serogroup 1 only)	Urine	Sterile container, RT, <24 h; 4ºC, >24 h -14 d
Anaerobes			
Bacteroides spp *Prevotella* spp *Fusobacterium nucleatum* *Peptostreptococcus* *Actinomyces* spp	Gram stain Anaerobic culture	Pleural fluid	Anaerobic transport vial, RT, 72 h; without transport RT ≤60 min
Mycobacteria			
Mycobacterium tuberculosis	Acid fast stain Mycobacterial Culture NAAT[b]	Pleural fluid	Sterile container, RT, 2 h; 4ºC, >2-24 h
	Histology	Pleural or lung biopsy Pleural fluid	Sterile container, RT, 2 h; 4ºC, 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; 4ºC, >2-24 h
Candida spp	As above plus may be evident on Gram stain	Pleural fluid	Sterile container, RT, 2 h; 4ºC, >2-24 h
Aspergillus spp	General fungal assays (i.e. stains, culture) plus galactomannan, (1-3)-□-D-glucan[b]	BAL	Sterile container, 4ºC, ≤5 d; -70ºC >5 d
	Galactomannan, (1-3)-β-D-glucan[c]	Serum	Clot tube RT, 2 d; 4ºC,
Histoplasma capsulatum	Fungal stain—calcofluor - KOH; other fungal stains Fungal culture Histology	Pleural fluid	Sterile container, RT, 2 h; 4ºC, >2-24 h
		Pleural biopsy	Sterile container, RT, 2 h; 4ºC, >2-24 h; formalin container for histology, RT 2-14 d
	Histoplasma antigen test[d]	Serum, urine, pleural fluid,	Clot tube, RT, 2 d; 4ºC, 2-14 d Sterile container (urine and fluid), RT 2 h; >2 – 72 h, 4ºC
	Histoplasma serum antibody (CF)	Serum	Clot tube RT, 2 d; 4ºC, 2-14 d
Coccidioides immitis/posadasii	Calcofluor white or other fungal stains, culture, plus histology	Pleural fluid Pleural biopsy	Sterile container, RT, 2 h; 4ºC, > 2-24 h
	Coccidioides serum antibody IgM (ID, LA, EIA) IgG antibody (CF, EIA)	Serum	Clot tube, RT, 2 d; 4ºC, 2-14 d
Blastomyces dermatitidis	Fungal stains and cultures plus histology	Pleural fluid Pleural biopsy	Sterile container, RT, 2 h; 4ºC, > 2-24 h
	Blastomyces antigen test[d]	Urine, pleural fluid, serum	4º C, ≤ 5 d

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Parasites			
Paragonimus westermani *Entamoeba histolytica* *Echinococcus* *Toxoplasma gondii*	Direct microscopic examination of pleural fluid and sputum for characteristic ova Direct examination of pleural fluid for trophozoite and cyst forms *Echinococcus* serum antibody testing Direct examination of pleural fluid for scolices[e] Giemsa-stained smear of pleural fluid or pleural biopsy	Pleural fluid Sputum Serum Pleural fluid	Sterile container, fresh samples 4º C, 60 min; RT, preserved samples > 60 min-30 d Clot tube, RT, 2 d; 4ºC, 2-14 d

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

[a] Sensitivity in non-bacteremic patients with pneumococcal pneumonia is 52-78%; sensitivity in bacteremic cases of pneumococcal pneumonia is 80-86%; specificity in adults is > 90%. Studies have reported a 21-54% false positive rate in children with NP carriage and no evidence of pneumonia and adults with chronic obstructive pulmonary disease [242, 253].

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

[c]Performance characteristics of these tests are reviewed in references [248, 249].

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

[e] Primary diagnosis of *Echinococcus* is usually made by imaging combined with serology. Scoleces in pleural fluid may be evident if the diagnosis was not considered prior to thoracentesis.

Table 30. Laboratory Diagnosis of Pulmonary Infections in Cystic Fibrosis

Etiologic Agents	Diagnostic Procedures	Optimum specimens	Transport Issues
Bacteria			
Staphylococcus aureus *Haemophilus influenzae* *Streptococcus pneumoniae* *Enterobacterales* *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia* *Achromobacter* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2-24 h, 4ºC
Burkholderia cepacia complex	Culture using *B.cepacia* selective agar	Throat swabs[a], expectorated sputum; other respiratory samples	Sterile container, RT, 2 h; >2-24 h, 4ºC
Opportunistic glucose non-fermenting gram-negative rods *Burkholderia gladioli* *Inquilinus* spp *Ralstonia* spp *Cupriavidus* spp *Pandorea* spp *Chryseobacterium* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2-24 h, 4ºC

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Herbaspirillum spp *Sphingobacterium spp*			
***Mycobacterium* spp**			
Mycobacterium abscessus	Mycobacteria culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory samples	Sterile container, RT, 2 h; >2-24 h, 4ºC
Mycobacterium avium complex	Mycobacteria culture		
Fungi			
Aspergillus spp allergic bronchopulmonary	Anti-Aspergillus IgE, IgG antibodies	Serum	Clot tube 4ºC, ≤5 d; >5 d, -70ºC
Aspergillus spp *Scedosporium apiospermum complex* *Lomentospora prolificans* *Apiotrichum mycotoxinivorans* *Exophiala dermatitidis* *Rasamsonia argillacea complex*	Galactomannan enzyme immunoassay Calcofluor -KOH or other fungal stain Fungal culture	Serum; BAL Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Clot tube 4ºC, ≤5 d; >5 d, -70ºC BAL fluid, sterile container, RT, 2 h; >2-24 h, 4°C Sterile container, RT, 2 h; >2-24 h, 4ºC
Viruses			
RSV	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 4ºC, 5 d; -70º C, >5 d
Influenza Adenovirus Rhinovirus Coronavirus including SARS CoV2 Parainfluenza virus Human metapneumovirus			

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

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

[b]Several US Food and Drug Administration-cleared NAAT platforms are currently available and vary in their approved specimen requirements and range of analytes detected. Readers should check with their laboratories regarding availability and performance characteristics, including certain limitations. See Table 26.

Table 31. Laboratory Diagnosis of Pneumonia in the Immunocompromised Host

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria			
See list of bacterial agents responsible for CAP and HAP above	**See Tables 21 and 22 above**	**See Tables 21 and 22 above**	**See Tables 21 and 22 above**
Additional bacterial pathogens of interest	Gram stain Culture	Expectorated sputum Bronchoscopically obtained specimens	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
Salmonella (non-typhoidal) *Elizabethkingia meningoseptica* *Listeria monocytogenes*			

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Nocardia and other aerobic Actinomycetes	Gram stain Modified acid fast stain Culture (include selective BCYE or other selective media)	Expectorated sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
Rhodococcus spp	Gram stain Culture		
Viruses			
Respiratory viruses	**See 22 and 23 above**	**See Tables 22 and 23 above**	**See Tables 22 and 23 above**
Cytomegalovirus	Quantitative NAAT[a]	Whole blood, plasma, BAL	EDTA tube, RT, 6-8 h; 4ºC>8-24 h Transport in viral transport media, 4ºC, 5 d; -70º C >5 d
	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, 4ºC, 5 d; -70º C >5 d
Herpes simplex virus	Culture combined with antigen detection; Use with cytologic analysis and or tissue histology for interpretation NAAT[a]	Bronchoscopically obtained specimens (protected brush) Lung tissue	Transport in viral transport media, 4ºC, 5 d; -70º C >5 d
***Mycobacterium* spp**			
M. tuberculosis	Acid fast stain AFB Culture NAAT Histology	Expectorated sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
M. avium intracellulare complex *M. kansasii* *M. xenopi* *M. haemophilum* Rapid growers e.g., *M. abscessus*	Acid fast stain AFB culture	Expectorated sputum Bronchoscopically obtained specimens	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h
	Histology	Lung tissue	Formalin container, RT, 2-14 d
Fungi			
Pneumocystis jirovecii[b]	DFA on BAL or sputum, (not tissue)	Expectorated sputum Induced sputum	Sterile cup or tube

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	NAAT[a] Cytologic stains (liquid samples)	Bronchoscopically obtained specimens	RT, 2 h; 4°C, >2 h-7 d
	Histology	Lung tissue	RT, 2 h; 4°C, >2-24 h Formalin container, RT, 2-14 d
Cryptococcus neoformans/gattii	Calcofluor or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens	Sterile cup or tube RT, 2 h; 4°C, >2-24 h
	Cryptococcal antigen test	Serum, 1 mL	Clot tube RT, 1 h; 4°C, >1 h-7 d
	Histology	Lung tissue	Formalin container, RT, 2-14 d Sterile container, RT, 2 h; 4°C, >2-24 h
Aspergillus spp	Calcofluor -KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Tissue	Sterile cup or tube RT, 2 h; 4°C, >2-24 h
	Galactomannan (1-3)-□-D-glucan	Serum BAL[b, c]	Clot tube 4°C, ≤5 d; >5 d, -70°C Sterile container for BAL RT, 2 h; 4°C, >2-24 h
	Histology	Lung tissue	Formalin container, RT, 2-14 d Sterile container, RT, 2 h; 4°C, >2-24 h
Fusarium spp *Scedosporium apiospermum*	Calcofluor -KOH; or other fungal stain Fungal culture	Expectorated sputum Induced sputum	Sterile cup or tube

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			RT, 2 h; 4ºC, >2-24 h
	Cytology or Histology	Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h Formalin container, RT, 2-14 d
	Fungal blood culture (see blood culture section)	Blood in aerobic blood culture bottle or specialized fungal medium (e.g. MycoF Lytic medium)	RT, 4 h
Mucorales (Zygomycetes) *Rhizopus*, *Mucor*, *Absidia* spp	Calcofluor -KOH or other fungal stain Fungal culture Histology	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile cup or tube RT, 2 h; 4ºC, >2-24 h Formalin container, RT, 2-14 d Sterile container, RT, 2 h; 4ºC, >2-24 h
Histoplasma capsulatum	Calcofluor- KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container RT, 2 h; 4ºC, >2-24 h
	Fungal blood culture (see blood culture section)	Blood in fungal blood culture bottle or lysis-centrifugation tube	RT, 4 h
	Histoplasma antigen test	Serum, urine, BAL, pleural fluid (if applicable)	Clot tube for serum RT, 2 d; 4ºC, 2-14 d; Sterile container for other samples 4º C, ≤5 d
	Histoplasma serology (CF)	Serum	RT, 2 d; 4ºC, 2-14 d
Coccidioides immitis/posadasii	Calcofluor -KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens	Sterile container RT, 2 h;

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		Lung tissue	4ºC, >2-24 h
	Coccidioides serum antibody IgM (ID, LA, EIA) IgG antibody (CF, EIA)	Serum	Clot tube RT, 2 d; 4ºC, 2-14 d
Enterocytozoon bieneusi (Microsporidiosis)	Histology Hematoxylin-eosin (H&E) stain; Warthin Starry stain; Giemsa stain; tissue Gram stain	Lung tissue	Formalin container, RT, 2-14 d
	Modified trichrome stain NAAT[d]	Induced sputum Bronchoscopically obtained specimens	Sterile container RT, 2 h; 4ºC, >2-24 h
Other endemic fungi	Calcofluor -KOH or other fungal stain Fungal culture	Expectorated sputum Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container RT, 2 h; 4ºC, >2-24 h
	Antigen test (Blastomyces)	Serum, urine, BAL, pleural fluid (if applicable)	Clot tube for serum RT, 2 d; 4ºC, 2-14 d; Sterile container for other samples 4º C, ≤5 d
Parasites			
Toxoplasma gondii	Microscopy—Giemsa stain smears (tissue) NAAT[a]	Induced sputum Bronchoscopically obtained specimens Lung tissue	Sterile container RT, 2 h; 4ºC, >2-24 h
	IgM antibody detection	Serum	Clot tube RT, 2 d; 4ºC, 2-14 d
		Induced sputum Bronchoscopically obtained specimens	Sterile container RT, 2 h; 4ºC, >2-24 h
Cryptosporidium	Modified acid-fast stain DFA NAAT[d]		
	Histology Hematoxylin-eosin (H&E) stain	Lung tissue	Formalin container, RT, 2-14 d
Strongyloides stercoralis	Microscopic wet mount examination of liquid	Induced sputum Bronchoscopically obtained specimens	Sterile container RT, 2 h;

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	samples for larval forms Culture (consult laboratory for availability)[e]		4°C,>2-24 h
	Histology	Lung tissue	Formalin container, RT, 2-14 d

Abbreviations: CAP, community acquired pneumonia; HAP, hospital acquired pneumonia; BCYE, buffered charcoal yeast extract; AFB, acid fast bacillus; DFA, direct fluorescent antibody test; EDTA, ethylenediaminetetraacetic acid; BAL, bronchoalveolar lavage; NAAT, nucleic acid amplification test; CF, complement fixation; GMS, Gomori methenamine silver stain; ID, immunodiffusion; IgG, immunoglobulin G: IgM, immunoglobulin M; LA, latex agglutination; EIA, enzyme immunoassay; RT, room temperature; VTM, viral transport medium

[a] Several US Food and Drug- cleared NAATs are currently available for the qualitative detection and quantification of herpes viruses and availability is laboratory specific. Several of the assays for quantification of cytomegalovirus use the World Health Organization international standard for reporting. Provider needs to check with the laboratory for optimal specimen sources, performance characteristics and turnaround time.

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

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

[0d] No US Food and Drug- cleared NAATs are currently available for testing respiratory specimens.

[e] Occasionally if *Strongyloides* is not suspected, larval tracks may be seen on agar plates containing bacteria.

IX. INFECTIONS OF THE GASTROINTESTINAL (GI) TRACT

Gastrointestinal infections include a wide variety of disease presentations as well as infectious agents. For many of these infections, particularly noninflammatory diarrhea and acute gastroenteritis of short duration, no laboratory testing is recommended [255, 256]. This section addresses the laboratory approach to establishing an etiologic diagnosis of esophagitis, gastritis, gastroenteritis and proctitis.

Key points for the laboratory diagnosis of gastrointestinal infections:

- The specimen of choice to diagnose diarrheal illness is the diarrheal stool, not a formed stool or a swab, with a notable exception in pediatrics where a swab is acceptable when feces is noted on the swab.
- Fecal testing for causes of infectious gastroenteritis using culture or culture independent methods is indicated for patients with moderate to severe, bloody, febrile, dysenteric, nosocomial, or persistent diarrheal illnesses or immunocompromised patients. Routine testing for other than *C. difficile* is often restricted in patients who have been hospitalized more than 3 days.
- Culture independent multiplex molecular tests are reported to be more sensitive than culture, result in higher rates of detection, and often cost more than culture methods.
- Toxin or nucleic acid amplification testing for *C. difficile* should only be done on diarrheal stool.

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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 32). Fungal microscopy with Calcofluor or KOH, examination by Gram stain of esophageal brushings, or histopathological examination of esophageal biopsies will establish the diagnosis of esophageal candidiasis in most cases. Fungal culture may be helpful in cases refractory to treatment. Determination of species may be helpful as *Candida glabrata* can be more resistant to treatment and *C. albicans* may develop resistance upon prolonged therapy. Histopathological examination is most reliable for diagnosis of viral esophagitis. Nucleic acid amplification test (NAAT) of esophageal biopsy or brushings has good sensitivity for HSV, while NAAT for CMV has poor sensitivity and specificity. Viral culture for CMV has little diagnostic utility [257].

Table 32. Laboratory Diagnosis of Esophagitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Candida spp	Calcofluor-KOH stain Fungus culture	Esophageal brushing or biopsy	Sterile container, RT, 2 h
	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2h-14 d
Herpex simplex virus	Histopathological examination	Esophageal biopsy	Formalin container, RT, 2h-14 d
	Nucleic acid amplification test (NAAT)	Esophageal brushing or biopsy	Closed container or viral transport device, RT, 2h
	HSV Culture	Esophageal brushing or biopsy	Viral transport device, on ice, immediately
Cytomegalovirus	Histopathological examination with immunohistochemical stain	Esophageal biopsy	Formalin container, RT, 2h-14 d

Gastritis

Helicobacter pylori is associated with atrophic gastritis, peptic ulcer disease and gastric cancer and is increasingly being found to be resistant to some of the antibiotics used to treat it. Diagnosis of *H. pylori* infection is critical as treatment can decrease morbidity. Testing is recommended for all patients with peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma and early gastric cancer [258]. In some patients with dyspepsia, non-invasive testing is an option [259]. Both invasive and noninvasive tests (Table 33) are available to aid in the diagnosis [260]. The test options are listed in priority order in the Table. However, the preferred test should be based on whether noninvasive or invasive specimens are collected and on the need or lack thereof for susceptibility testing. 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 two weeks prior to collection and as such, pose greater risks to the patient.

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Culture, although not routinely performed, allows for antimicrobial susceptibility testing. However, the organism is fastidious, growth in culture routinely requires at least 7 days of incubation. Delayed transport may adversely affect recovery. If cultures cannot be setup immediately, the laboratory should be contacted for optimal transport medium and transport conditions. Two biopsies from antrum and two biopsies from posterior corpus are recommended for histopathologic examination. Rapid urease tests can be performed on a single biopsy from antrum and a single biopsy from the posterior corpus. Performance of both histopathological examination and rapid urease testing improves the sensitivity of invasive testing [260]. If susceptibility testing is not required, culture is not necessary, eliminating the need for collection of additional specimens. The advantage to the noninvasive assays such as the urea breath test and stool antigen or NAAT determinations is that patients can avoid endoscopy and gastric biopsy [258-260]. The urea breath test is performed in the clinic. This assay has a sensitivity of ~95%, comparable to the invasive assays. Most stool antigen tests have a reported sensitivity of 90-98%. The noninvasive assays are also useful to test for organism eradication after therapy; the urea breath test and stool antigen tests have comparable sensitivity. Stool NAAT can be used to detect and simultaneously assess for clarithromycin resistance in *H. pylori.* Serodiagnosis has a lower sensitivity (<90%) and specificity (90%), is not recommended for initial diagnosis nor is it useful for test of cure after therapy as it can remain positive years after infection.

Table 33. Laboratory Diagnosis of Gastritis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Helicobacter pylori	*H. pylori* stool antigen test	Stool specimen	Closed container, RT, 2 h
	Urea breath test[1]	Radiolabeled breath	Special collection device
	Agar-based or rapid tissue urease tests[2]	One biopsy from antrum and one biopsy from posterior corpus	Closed container, RT, immediately, or direct inoculation
	Histopathological examination[3]	Two biopsies from antrum and two biopsies from posterior corpus	Formalin container, RT, 2-14 d
	H. pylori culture[3]	One biopsy from antrum and one biopsy from posterior corpus	Sterile container(if setup immediately), RT, Transport medium such as Brucella broth with 20% glycerol or as recommended by laboratory (if delayed), 2-8 C
	H. pylori stool PCR with detection of antimicrobial resistance	Stool specimen	Closed container, transport medium, RT, 7 days

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

[2]Agar-based or rapid urease tests have a slightly lower sensitivity of 90 – 95% compared to histopathological examination but offer the advantage of providing rapid results. They may be performed point-of-care or in the

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laboratory. When these tests are performed on gastric fluid, orogastric brush or “string” specimens, they have lower sensitivity than when performed on biopsy specimens.
[3]Culture of properly collected and transported biopsy specimens, has a sensitivity of 95% as does histopathological examination. Culture may not be routinely available but allows for antimicrobial susceptibility testing.

Gastroenteritis, infectious and toxin-induced diarrhea

Gastrointestinal infections encompass a wide variety of symptoms and recognized infectious agents (Table 34). 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. For most patients with acute diarrhea, diagnostic testing is not indicated . However, fecal testing, using culture or culture independent methods, is indicated for patients with moderate to severe, bloody, febrile, dysenteric, nosocomial, or persistent diarrheal illnesses [255, 256, 261] or for immunocompromised patients. Insurance coverage may be limited for highly multiplexed culture independent molecular assays (12 or more targets) when performed on outpatients not meeting these indications. Clinical management in this patient population is often not affected as most infections are self-resolving. Communication with the laboratory is encouraged to determine what organisms, methods, and screening parameters are included as part of the routine enteric pathogen culture or culture independent method. Most laboratories will have the ability to culture for *Salmonella, Shigella, Campylobacter*, and test for Shiga toxin-producing *E. coli*. Culture independent methods are often routinely available for *Clostridioides difficile* and may be available for other bacterial, viral, and protozoic causes of gastrointestinal infections. *C. difficile* testing should be limited to patients with new onset diarrhea who are not taking laxatives with typical clinical presentation and risk factors. Although often included in multiplex molecular panels, singleplex testing is adequate. Stool culture has longer time to result and often fails to detect the causative agent. Thus, when available, culture-independent methods are recommended for detection of bacterial pathogens [255, 256, 261, 262]. Viral gastroenteritis is often self-resolving, thus multiplex panels targeting viruses often have little clinical impact. However, they are recommended for immunocompromised patients and for infection control purposes [263, 264]. Testing for parasites should be considered in patients with diarrhea persisting for longer than 7 days. Multiplexed molecular panels are limited as to the parasitic agents detected.

The specimen of choice is the diarrheal stool (i.e., takes the shape of the container). Multiple stool specimens are rarely indicated for the detection of bacterial stool pathogens. In studies of adult patients who submitted more than one specimen, the bacterial enteric pathogen was detected in the first sample 87 – 94% of the time, with the second specimen bringing the positive rate up to 98% [265]. In pediatric patients, the first specimen detects 98% of the enteric pathogens [266]. Thus, one sample for children and a second for selected adult patients may be considered. Rectal swabs are less sensitive than stool specimens when culture methods are employed and are not recommended for culture from adults but in symptomatic pediatric patients’ rectal swabs and stool specimens are equivalent in the ability to detect fecal pathogens [267, 268]. Rectal swabs have been shown to be as sensitive as stool specimens when culture independent methods are employed

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[269], although no tests are FDA-cleared for their use. Due to low clinical yield, routine enteric pathogen testing, other than for *C. difficile,* is often restricted on patients who have been hospitalized greater than 3 days [270, 271].

Table 34. Laboratory Diagnosis of Gastroenteritis, Infectious and Toxin-induced Diarrhea

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria			
Clostridioides difficile	Glutamate dehydrogenase (GDH) antigen and NAAT or toxin A/B performed as part of an algorithm	Unformed Stool	Closed container, RT, 2 h
	NAAT and toxin A/B	Unformed Stool	Closed container, RT, 2 h
	Nucleic acid amplification test (NAAT)	Unformed Stool	Closed container, RT, 2 h
Salmonella spp *Shigella* spp *Campylobacter* spp	NAAT	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair or manufactured specified transport medium, RT, 24 h
	Routine stool enteric pathogen culture[1]	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair transport medium, RT, 24 h
Enterohemorrhagic *E. coli* (including *E coli* O157:H7 and other Shiga-toxin-producing *E. coli*)	NAAT for Shiga toxin genes	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair or manufactured specified transport medium, RT, 24 h
	Shiga-toxin immunoassay	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair transport medium, RT, 24 h
	Culture for *E. coli* O157:H7[3]	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair transport medium, RT, 24 h
Yersinia enterocolitica *Vibrio* spp *Plesiomonas shigelloides* *E. coli* Enterotoxigenic Enteroinvasive[5] Enteropathogenic[5] Enteroaggregative[5]	NAAT[4]	Unformed Stool	Closed container, RT, 2 h[2]
Yersinia enterocolitica *Vibrio* spp *Aeromonas* spp *Plesiomonas shigelloides* *Edwardsiella tarda*	Specialized stool cultures[6]	Unformed Stool	Closed container, RT, 2 h[2] Cary-Blair transport medium, RT, 24 h
Bacillus cereus *Clostridium perfringens* *Staphylococcus aureus*	Specialized procedure for toxin detection[7]	Stool	Closed container, RT, 2 h

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Clostridium botulinum	Mouse lethality assay, specialized culture, and toxin detection[8] (Usually performed at the State Public Health Laboratory)	Stool, enema (infant botulism), gastric contents, vomitus[9]	Closed container Store and transport specimens at 4°C. **Do not freeze**
Parasites			
E. histolytica/dispar *Blastocystis hominis*[10] *Dientamoeba fragilis* *Balantidium coli* *Giardia lamblia* Nematodes including: *Ascaris lumbricoides, Strongyloides stercoralis*[11], *Trichuris trichiura,* Hookworms Cestodes (Tapeworms) Trematodes	Ova and parasite examination including permanent stained smear	Stool. Three consecutive stool samples within ten days improves the chances for detection.[12]	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
E. histolytica	*E. histolytica* species specific immunoassay	Stool	Stool not in fixative
	NAAT[4]		Cary-Blair transport, RT, 24h
Giardia lamblia[13] *Cryptosporidium* spp[13]	Direct fluorescent immunoassay	Stool	Stool in fixative, 2-24h
	Enzyme immunoassay	Stool	Stool in fixative, 2-24h
	NAAT[4]		Cary-Blair transport, RT, 24h
Coccidia including *Cryptosporidium, Cyclospora, Cystoisospora*	Modified acid fast stain[14] performed on concentrated specimen	Stool. At least 3 stool specimens collected on subsequent days need to be examined before coccidial infection can be ruled out	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
Cryptosporidium[4], Cyclospora	NAAT[4]	Stool	Cary-Blair transport, RT, 24h
Microsporidia	Modified trichrome stain[14] performed on concentrated specimen	Stool. At least 3 stool specimens collected on subsequent days need to be examined before microsporidial infection can be ruled out	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
	Histologic examination with EM confirmation	Small bowel biopsy	Formalin container, RT. 2-14d
Enterobius vermicularis	Pinworm paddle or tape prep	Perianal area. 4-6 consecutive	RT, 2 h

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		negative tapes are required to rule out a pinworm infection	
Virus			
Astrovirus[14,15] Calicivirus[14,15] (Norovirus, Sapovirus) Enteric Adenovirus[15] Rotavirus[14,15] Enterovirus/Parechovirus[15]	NAAT	Stool	Closed container, RT, 2 h
Rotavirus Enteric Adenovirus	Enzyme immunoassay	Stool	Closed container, RT, 2 h
Cytomegalovirus	Histopathological examination	Biopsy	Formalin container, RT, 2-14 d
	CMV Culture	Biopsy	Sterile container, RT, immediately

[1]A routine stool culture in most laboratories is designed to detect *Salmonella* spp, *Shigella* spp, and *Campylobacter* spp *E. coli* O157 or Shiga-toxin producing *E. coli* may or not be included.

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

[3]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.

[4]Available as part of some multiplex panels

[5]The clinical relevance of these organisms is controversial. They are rarely associated with diarrhea in industrialized countries.

[6]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.

[7]*Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus* cause diarrheal syndromes that are toxin mediated. Testing has no clinical necessity as symptoms are short-lived. Testing is only performed for food-borne outbreaks. 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.

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

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

[10]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.

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

[12] A second specimen should be submitted only when the first is negative and the patient remains symptomatic, with a third specimen being submitted only if the patient continues to be negative and symptomatic

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[13] *Cryptosporidium* and *Giardia lamblia* testing is often offered and performed together as the primary parasitology examination. Additional parasite examinations should follow if a travel history, risk factors or clinical symptoms suggest parasitic disease.
[14] These stains may not be routinely available.
[14] Also available as part of some multiplex panels, however testing is not routinely recommended except in immunocompromised patients, infection control purposes, or outbreak investigations due to lack of clinical impact.
[15] Asymptomatic shedding is common [272-274].

Stool culture

Stool culture is indicated for detection of invasive bacterial enteric pathogens when pathogens of high clinical or public health importance are present. Culture methods must be used for test of cure. When culture independent methods are used with the detection of reportable agents, specimens should either be cultured to recover the isolate or the stool provided to Public Health Laboratories to culture, for epidemiologic follow up.

When culture methods are employed, most laboratories routinely detect *Salmonella, Shigella* and *Campylobacter* and, more recently, Shiga toxin-producing *E. coli* in all stools submitted for culture. *Salmonella* spp can take 24 – 72 hours to recover and identify to genus alone with the specific serotyping usually performed at the State Public Health Laboratory level. It is recommended that a test for the detection of Shiga toxin, with or without detection of *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 which 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 [275]. Screening algorithms which limit testing to bloody stools may also miss both O157 and non-O157 isolates. Screening of stool for toxin producing *E. coli* is recommended for all pediatric patients.

Detection of *Vibrio* and *Yersinia* species in the U.S. 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 whole genomic sequencing for national surveillance purposes.

Culture independent methods

Culture independent methods are becoming increasingly available. Nucleic acid amplification assays vary from singleplex to highly multiplexed assays. It is imperative to communicate with the laboratory to determine what organisms are detected. Culture independent methods can detect pathogens in as little as one to 5 hours compared to the 24 – 96 hours often required for culture. These assays are reported to be more sensitive than culture and have resulted in much higher rates of detection [261]. Highly multiplexed assays allow for the detection of mixed infections where the importance of each pathogen is unclear. They may also allow for the detection

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of pathogens, such as enteroaggregative or enteropathogenic *E. coli* or viruses where clinical significance and the indication for therapy is unclear. Culture independent methods should not be used as test of cure as they will detect both viable and non-viable organisms.

Clostridium botulinum

Botulism is an intoxication in which a protein exotoxin, botulinum toxin, produced by *Clostridium botulinum* causes a life-threatening flaccid paralysis. Diagnosis, while not usually confirmed by the hospital microbiology laboratory, is made by clinical criteria, allowing prompt initiation of essential anti-toxin therapy. The microbiologic diagnosis is dependent upon 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/vomitus (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 [276]. Testing is available through the Center for Disease Control and Prevention. Notify the laboratory if botulism is suspected.

Clostridioides difficile

Numerous methods have been employed for the laboratory diagnosis of infection caused by *Clostridioides difficile*. Toxigenic culture is the gold standard assay for the detection of *C. difficile* [277]. It is slow and labor intensive and not routinely performed in the 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 or immunochromatographic methods has been performed. These tests are significantly faster with results available in less than 2 hours. However, they have variable sensitivity and perform poorly compared to the reference methods. Utilization of an assay that detects both toxin A and toxin B improves the sensitivity. These tests should be combined with a highly sensitive screening test such as glutamate dehydrogenase or NAAT [278]. Glutamate dehydrogenase (GDH) antigen assays are sensitive, have poor specificity but high negative predictive value. Nucleic acid amplification assays for the detection of *C. difficile* have reported sensitivity of 93 – 100% but suffer from poor positive predictive value for identification of patients with *C. difficile*-associated disease due to detection of *C. difficile* in colonized patients. NAAT detects viable and nonviable organisms as well as non-toxin producing organisms. When performed on unformed stool from symptomatic patient, NAAT alone may be adequate [279]. A two or three step algorithm allows for optimal identification of patients with *C. difficile*-associated colitis [277, 279-282]. To reduce turn-around time, reduce costs and to improve accuracy of diagnosis of *C. difficile*-associated disease, some laboratories employ an algorithm that utilizes the GDH as a rapid screening test, followed by toxin A and B detection, cytotoxin testing and/or NAAT. This algorithm allows for both the rapid reporting of most negative specimens and the specificity of cytotoxin testing or NAAT. To decrease the identification of colonized patients, some laboratories are utilizing

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algorithms that utilize both NAAT tests and tests to detect toxin. This algorithm allows for the identification of patients most likely to be colonized (NAAT +, toxin -) and patients at highest likelihood of *C. difficile*-associated disease (NAAT+, toxin +) [283, 284].

Testing should only be performed on patients with diarrhea, defined as $\geq$ 3 unformed stools within 24 hours. 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 [280, 285].

Testing should only be performed in children older than 2 years of age. Toxigenic *C. difficile* colonizes nearly 50% of infants in the first year of life with asymptomatic rates approaching those of healthy adults around 2 years of age. However, there is data to suggest that *C. difficile* may be the cause of disease in some infants. Thus, children less than 1 year of age should not be tested as the detection of the organism and toxin does not differentiate colonization from infection [286]. For children 1-2 years of age, testing for other causes should be pursued first with *C. difficile* testing being performed only if there is no alternative cause and the symptoms are severe or the clinical presentation is consistent with *C. difficile* infection [281, 287].

Since 2000, an increase in *C. difficile*-associated disease with increased morbidity and mortality has been reported in the United States, Canada and the UK. 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 and is resistant to multiple antibiotics. A commercially available FDA-cleared NAAT for binary toxin and the tcdC deletion genes identifies this strain. The test may be useful for epidemiological purposes; however, the predominance of this strain has decreased over time [288, 289] and other strains have the same potential for hypervirulence.

Parasites

The number of specimens to be submitted for parasitologic examination may be a controversial subject [290, 291]. Historically, when using conventional microscopic procedures, it was recommended that three specimens collected over a 7-10 day period be submitted for ova and parasite (O&P) examination. Options for cost-effective testing today include examination of a second specimen only when the first is negative and the patient remains symptomatic, with a third specimen being submitted only if the patient continues to be O&P negative and symptomatic. Targeted use of immunoassay testing or NAAT for the most common parasites based on geography, patient demographics, and physician request, can also be used as a screen with only negative patients with continued symptoms or patients with specific risk factors requiring full O&P

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examination. Immunoassays for *Giardia* may be sensitive enough that only a single specimen is needed. However, consider additional samples if there is a high level of clinical suspicion for *G. lamblia* infection and infection with other intestinal parasites is low [292, 293]. No data is available on the number of specimens required to rule out infection when NAAT is performed. NAAT are available as a component of some multiplexed assays.

The specimen preservative to be employed, often supplied by the laboratory, depends on the need to perform immunoassay procedures or special stains or NAAT on the specimens and the manufacturer's recommendations for specimen fixative or preservative. It is imperative that the laboratory be consulted to assure proper transport conditions are utilized. Polyvinyl alcohol (PVA) is the gold standard for microscopic examination, however due to the presence of mercuric chloride, modifications which 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* [294]. Only an immunoassay or NAAT can differentiate these organisms.

Traditional microscopy-based examination of fecal specimens continues to be the predominant method employed for detection of fecal parasites. Concentration of stool specimens, slide preparation most commonly employing trichrome staining, and microscopic examination is a predominantly manual method requiring significant technologist time and expertise. Recently, slide scanners have been developed to digitize the images and systems employing artificial intelligence (AI) have been developed for fecal trichrome slide interpretation. While early studies have shown increased sensitivity and increased throughput [295], a requirement for confirmation of images by technologists remains [296].

Viruses

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

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 [297]. Since the beginning of 2022, monkeypox virus, causing the disease now known as mpox, has been detected in nonendemic parts of the world and recognized as a cause of proctitis [298]. One sample is usually sufficient for diagnosis (Table 35).

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Table 35. Laboratory Diagnosis of Proctitis

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Neisseria gonorrhoeae	NAAT[1]	Rectal swab	Transport is manufacturer dependent
	Routine aerobic culture employing media for the recovery of *N. gonorrhoeae*	Rectal swab	Swab in Amies or Stuart's transport medium, RT, 8 h
Chlamydia trachomatis	NAAT[1]	Rectal swab	Transport is manufacturer dependent
Herpes simplex virus	Viral culture	Rectal swab	Viral transport medium, RT, 2 h, wet ice if >2 h
Monkeypox virus[2]	NAAT	Rectal swab	Viral transport medium, RT, 2 h, wet ice if >2 h
Treponema pallidum	RPR or VDRL with confirmatory *T. pallidum* specific test or syphilis IgG	Serum	Clot tube, RT, 2 h

[1]NAAT – nucleic acid amplification test. 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 turnaround time.
[2] Not all laboratories that offer monkeypox testing will offer testing on rectal swabs.

X. 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 intra-peritoneal abscesses, hepatic and splenic abscesses, pancreatitis, and biliary tract infection. As molecular analyses begin to be used to define the microbiome of the gastrointestinal and genitourinary tract, contemporary culture protocols will surely evolve to accommodate new, emerging information. The future use of gene amplification and sequencing for identification of microorganisms in these infections will most likely show that for every organism currently identified by culture there will be several times that number that cannot be cultivated using current technologies. To remain focused on contemporary methods currently available in the diagnostic microbiology laboratory, the tables outline the most likely agents of each entity (Table 36) and how best to evaluate the situation with existing techniques (Table 37).

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

Key points for the laboratory diagnosis of intraabdominal infections:
o Most importantly, the laboratory needs the specimen--not a swab of the specimen.

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- 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. Depending on clinical suspicion, these two samples may be submitted in a single vial or submitted as two separate specimens.
- Pus alone may not reveal the etiologic agent on Gram or other direct smears 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

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 pre-existing risk factors such as cirrhosis with ascites [299, 300]. SBP is an ascitic fluid infection without an evident intraabdominal focus. It tends to be monomicrobic and caused by aerobic organisms from the intestinal tract, therefore anaerobic cultures are less valuable. Sufficient fluid (e.g. at least 10 mL and up to 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 36). Bacterial culture would be the recommended approach. Alternately, because SBP and infections of ascites fluid tend to be monomicrobic, an aerobic blood culture bottle can be inoculated with fluid (volume dependent upon blood culture system) if the presence of a single organism is reasonably certain. A Gram stain of the fluid can provide critical information and should be done prior to blood bottle 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 either at the bedside or in the laboratory with the fluid. Mass spectrometry, sequencing and 16S ribosomal RNA gene PCR/sequencing can be used to identify isolates present in these specimens if these techniques are available to the laboratory. If more than one morphologic type is noted in the Gram stain, a blood bottle or other 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

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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*.

Secondary peritonitis

The diagnosis of secondary peritonitis is dependent upon identifying a source for invading microorganisms - usually genitourinary or gastrointestinal microbiota [300, 301]. There are numerous causes of secondary peritonitis including iatrogenic or accidental trauma, perforated appendix or diverticuli, typhlitis, or intra-abdominal abscess. Complications from bariatric surgery may also cause secondary peritonitis. Unlike SBP, however, secondary peritonitis tends to be polymicrobic and may include anaerobic microbiota. 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 microbiota (*Clostridium* spp, *Enterococcus* spp, and less commonly *Bifidobacterium* spp and *Peptostreptococcus* spp). Infectious complications following bariatric surgery are frequently due to Gram-positive cocci and yeast (*Candida* spp) Since many obese patients have had prior exposure to antibiotics, multidrug resistant organisms are of concern [302-304]. 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. If a parasitic etiology is suspected after other etiologies are ruled out, a request for ova and parasite examination could be considered.

Peritoneal fluid should be sent to the laboratory in an anaerobic transport system for Gram stain and aerobic and anaerobic bacterial cultures. Inoculation of blood culture bottles alone with peritoneal fluid is not appropriate in this setting, as competitive bacterial growth in broth cultures could mask the recovery of clinically important pathogens (Table 36). 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 polymicrobic nature of secondary peritonitis, clinicians should not expect or request identification and susceptibility testing of all organisms isolated. Rather, when three or more species are isolated, the laboratory should provide a general description of the culture results (e.g., mixed aerobic and anaerobic intestinal microbiota) with selective identification of recognized pathogenic organisms such as MRSA, beta-hemolytic *Streptococcus* spp, multi-drug-resistant organism (with patient history), vancomycin-resistant *Enterococcus* spp (VRE), etc.) to guide empiric antimicrobial therapy [299, 300, 305]. The clinician can call the laboratory to

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discuss the culture results and perhaps help guide further workup, but identifying everything will likely not be an option. 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.

Tertiary peritonitis

This entity refers to persistent or recurrent peritonitis following unsuccessful treatment of secondary peritonitis. Tertiary peritonitis might also indicate the presence of an intra-abdominal abscess or organisms that are refractory to broad spectrum antimicrobial therapy such as VRE, *Candida* species, *Pseudomonas aeruginosa*, or biofilm-producing bacteria like coagulase-negative *Staphylococcus* spp. Fluid cultures from cases of tertiary peritonitis are commonly negative for bacteria [299]. In any case, cultures appropriate for spontaneous or secondary peritonitis may be helpful (Table 37). The possibility of infection caused by unusual or slowly growing organisms such as filamentous fungi and *Mycobacterium* spp should be entertained if routine 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 as initial orders.

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 monomicrobic and rarely anaerobic. In the case of PDAP, however, the list of likely suspect organisms is quite different from SBP. Gram-positive bacteria (predominantly *Staphylococcus* spp and to a lesser extent, *Streptococcus* and *Corynebacterium* spp) account for > 60% of cultured microorganisms. Gram-negative bacteria, (mostly *E. coli, Klebsiella,* and *Enterobacter* spp) represent < 30% of positive cultures while anaerobes comprise < 3% of isolates [300, 306, 307]. Fungi, especially *Candida* species contribute to the same number of identified infections as anaerobes [308]. Cultures can remain negative in >20% of all cases of PDAP [308]. 10 – 50 mL of dialysate should be collected for concentration and culture, cytospin Gram stain evaluation, analysis for protein, cell count and differential (Table 37). Peripheral blood cultures are rarely positive in cases of PDAP [300]. 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 [306, 308]. 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.

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

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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 [309-311]. In regions where *E. histolytica* disease is endemic, the use of serology or serum antigen detection tests can be helpful to exclude amebic abscess [312] whereas examination of stool for cysts and trophozoites is generally not (Table 37). In cases where amebic disease or *Echinococcus* infection is highly suspected, the disease must be investigated carefully with imaging and serology before specimens are collected. Surgical treatment and specimen collection must be conducted with extreme precaution to avoid further spread of the disease. 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. Ideally, the wall of the abscess and the abscess contents should be sampled when possible since an aggressive infection includes the tissue of the wall. Commonly recovered isolates include *Klebsiella* spp, *E. coli,* and other Enterobacterales*, Pseudomonas* spp, *Streptococcus* spp including *Streptococcus anginosus* group, *Enterococcus* spp, viridans group *Streptococcus*, *S. aureus*, *Bacteroides* spp, *Fusobacterium* spp (especially with delayed treatment of Lemierre's syndrome), *Clostridium* spp, and rarely *Candida* spp [309-311]. Aerobic and anaerobic bacterial culture should be requested (Table 37). Blood cultures can also be helpful in establishing an etiology if collected prior to the institution of antimicrobial therapy [310, 311]. 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).

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 36). Parasitic causes include *Ascaris* and *Clonorchis* spp or any parasite that can inhabit the biliary tree leading to obstruction [309]. At a minimum, cultures for aerobic bacteria (anaerobes if the aspirate is collected appropriately and transported in an appropriate container) and Gram stain should be requested. In addition to plate cultures, fluids may be inoculated into blood culture bottles but pathogens may be obscured in polymicrobial infections. When signs of sepsis and peritonitis are present, blood and peritoneal cultures should be obtained as well.

For patients with HIV infection, the list of potential agents and subsequent microbiology evaluations needs to be expanded to include *Cryptosporidium*, microsporidia, *Cystoisospora* (*Isospora) belli,* CMV, and *Mycobacterium avium* complex [309]. 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.

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Splenic abscess

Most cases of splenic abscess are the result of metastatic or contiguous infectious processes, trauma, splenic infarction, or immunosuppression [312]. Infection is most likely aerobic and monomicrobic with *Staphylococcus* spp, *Streptococcus* spp, *Enterococcus* spp, *Salmonella* spp and *E. coli* commonly isolated. Anaerobic bacteria have been recovered in 5 – 17% of culture-positive cases [313]. Aspirates should be processed in a similar manner as pyogenic liver abscesses including aerobic and anaerobic culture, Gram stain, and concomitantly collected peripheral blood cultures (Table 37). 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) [314]. 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.

Secondary pancreatic infection

Most cases of acute or chronic pancreatitis are produced by obstruction, autoimmunity or alcohol ingestion [315, 316]. Necrotic pancreatic tissue generated by one of these processes can serve as a nidus for infection [315, 316]. Infectious agents associated with acute pancreatitis are numerous and diverse, however, superinfection of the pancreas is most often caused by gastrointestinal microbiota such as *E. coli, Klebsiella* spp and other members of the Enterobacterales, *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 peripheral blood cultures (Table 37). Antimicrobial susceptibility results from isolated organisms can be used to direct therapy to reduce the likelihood of 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.

Table 36. Etiologic Agents Involved in Intra-abdominal Infections

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	Enterobacterales	Gram-negative; oxidase-positive rods	Gram-negative nonfermenters	Gram-positive cocci	Gram-positive rods	Anaerobes	*N. gonorrhoeae*	*C. trachomatis*	*Mycobacterium* 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			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 37. Specimen Management for Intra-abdominal Infections

Condition	Diagnostic Procedure	Optimum Specimen	Transport Issues
Spontaneous Bacterial Peritonitis/Ascites; Secondary Peritonitis; Tertiary Peritonitis; Peritoneal Dialysis-associated Peritonitis	Aerobic and anaerobic[1] culture: Gram stain	10-50 mL peritoneal fluid and Sample in blood culture bottle[1]	RT; if >1 h, 4°C
	Peripheral blood culture	2-3 sets blood culture bottles	RT, do not refrigerate

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	AFB stain and culture *Mycobacterium* NAAT[2]	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[3]	Stool, peritoneal fluid, bile, duodenal aspirate	Transport stool in parasite transport vial; others <1 h at RT
Space-Occupying Lesions of the Liver	Aerobic and anaerobic culture Gram stain	Lesion aspirate	Anaerobic transport; RT, if >1 h, 4□C
	Blood culture	2-3 sets in blood culture bottles	RT, do not refrigerate
	Cultures for *N. gonorrhoeae* and *C. trachomatis*	Lesion aspirates *C. trachomatis* specimen may include swab of liver capsule or surrounding peritoneum	For *N. gonorrhoeae:* Amies charcoal transport, RT. For *C. trachomatis:* Chlamydia transport medium at 4°C
	NAAT *for N. gonorrhoeae* and *C. trachomatis*	Urethra, pelvic specimen (approved swabs), or urine (sterile cup)	RT for <1 h or 4□C
	Fungal culture and KOH or calcofluor white microscopy	10-50 mL fluid	RT, if >1 h, 4□C
	Serology for *E. histolytica*	Serum	Clot tube, RT, 2 h
	Antigen detection for *E. histolytica*	Liver aspirate	RT for <30 min, then 4□C. Freeze (-20°C) if shipping to reference laboratory
Infections of the Biliary Tree	Aerobic and anaerobic culture Gram stain before culture	Aspirate from lesion	Anaerobic transport device; RT, if >1 h, 4□C
	Blood culture	2-3 sets	RT; do not refrigerate

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	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 *E. histolytica*	Serum	RT for <30 min, then 4□C. Freeze (-20°C) if shipping to reference laboratory
Splenic Abscess	Aerobic and anaerobic culture Gram stain	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4□C
	Blood culture	2-3 sets	RT; do not refrigerate
	AFB stain and culture *Mycobacterium* NAAT can be done[2]	Fluid or tissue	RT. If >1 h, 4□C
	Fungal culture and KOH or calcofluor white microscopy	10-50 mL of aspirate or tissue	RT. If >1 h, 4□C
	Serology for *Entamoeba* and *Echinococcus*	Serum	RT for <30 min, then 4□C. Freeze (-20°C) if shipping to reference laboratory.
Secondary Pancreatic Infections	Aerobic and anaerobic culture Gram stain prior to culture	Aspirate from lesion	Anaerobic transport at RT. If >1 h, 4□C.
	Blood culture	2-3 sets	RT; do not refrigerate
	Fungal culture and KOH - calcofluor microscopy	10-50 mL aspirate or tissue	RT; if >1 h, 4□C

AFB, acid-fast bacillus; NAAT, nucleic acid amplification test; CMV, cytomegalovirus

[1] In the laboratory, if Gram stain reveals multiple morphologies of organisms, do not inoculate blood culture bottles with the fluid as competitive bacterial growth could mask the recovery of clinically significant pathogens. If fluid is

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inoculated into blood culture bottles, a conventional culture must also be used. Anaerobic cultures of peritoneal fluid are only necessary in cases of secondary peritonitis.
[2]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 non-respiratory samples.
[3]Procedure to be used in cases of secondary peritonitis in appropriate clinical situations.

XI. URINARY TRACT INFECTIONS

Clinical microbiology tests of value in establishing an etiologic diagnosis of infections of the urinary tract are covered in this section which includes specimens and laboratory procedures for the diagnosis of cystitis, pyelonephritis, prostatitis, epididymitis and orchitis.

Key points for the laboratory diagnosis of urinary tract infections:

- In the absence of signs and symptoms consistent with urinary tract infection, a urine culture is typically not recommended
- Urine collected for culture should not be kept at room temperature for more than 30 minutes. Hold at refrigerator temperatures or utilize a preservative tube if not processed by the laboratory within 30 minutes.
- Reflexing to culture based on a positive pyuria screen may be considered. If a reflexive algorithm is implemented, it should be a locally approved policy guided by clinicians and laboratorians
- Three or more species of bacteria in a urine specimen usually indicates contamination at the time of collection and interpretation remains challenging
-

IDSA guidelines for diagnosis and treatment of urinary tract infections are published [317] as are ASM recommendations [318]. These guidelines provide diagnostic recommendations that are similar to those presented here (Table 38). The differentiation of cystitis and pyelonephritis requires clinical information and physical findings as well as laboratory information, and from the microbiology laboratory perspective, the spectrum of pathogens and diagnostic approach are similar for the two syndromes [319]. Urine culture is one of the most commonly ordered diagnostic tests in the 'work up' of infectious diseases, and its effectiveness relies on a multimodal and multi-disciplinary diagnostic stewardship [320]. In the absence of signs and symptoms consistent with urinary tract infection, primarily dysuria, urgency, frequency, and/or costovertebral tenderness, a urine culture is typically not recommended, regardless of the presence of pyuria, except in specific patient populations. There is no single laboratory parameter or combination of parameters that can sufficiently predict whether the patient has a urinary tract infection or if bacteria will be recovered in culture. Recent studies have demonstrated that pyuria alone is likely the best marker in terms of negative predictive value, and culturing urine samples with demonstrated pyuria, including markers such as leukocyte esterase or white blood cells (WBCs), increases the likelihood of a positive culture; however, methods for detection of pyuria (both

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WBCs and leukocyte esterase) have not been standardized, and variable results can be seen due to the use of spun vs. unspun urine, different lengths of centrifugation, and utilization of an automated vs. manual system (i.e. 'dipstick') [321, 322]. While >10 WBCs per low power field is a commonly adopted threshold for a urinalysis with reflex to culture (UARC) algorithm, this threshold cannot be effectively applied to all patient groups, and exceptions are recommended for specific groups including patients who are pregnant and patients with urologic abnormalities or undergoing urologic procedures [321]. Laboratory algorithms should take into account local patient populations (patients with neutropenia, pediatric patients, patients with a high prevalence of asymptomatic bacteriuria or non-specific pyuria, such as patients with indwelling catheters, etc.) and should be guided by both clinicians and laboratorians. In older adults with mental status changes or falls and without classic localizing UTI symptoms, bacteriuria may be unrelated, and a thoughtful approach should be taken before including urine culture as part of the diagnostic plan [317].

Cystitis and pyelonephritis

Laboratory culture of urine continues to be routinely performed using quantitative methods, with the standardized threshold in symptomatic patients of >100,000 CFU/ml for organisms identified as common pathogens, however, many sites now use either 10,000 CFU/ml or 1000 CFU/ml threshold based on the method of collection or patient population as a baseline for culture workup and clinical significance [323]. Laboratories typically provide antimicrobial susceptibility testing (AST) for potential pathogens recovered in significant numbers as part of standard laboratory protocol. The Gram stain is not routinely utilized for the diagnosis of uncomplicated urinary tract infection, but it may have limited utility in patients with febrile urinary tract infection (pyelonephritis, prostatitis, urosepsis) where high numbers of Gram-negative bacilli may be present in urine [324].

Because urine may be readily contaminated with urogenital microbiota, proper specimen collection is essential for urine culture and should be performed using a process that minimizes contamination from the perineal and superficial mucosal microbiota. Although some literature suggests that traditional skin cleansing in preparation for the collection of midstream voided or “clean catch” specimens is not of benefit, no recommendation against cleansing can be made due to lack of sufficient evidence; therefore, skin cleansing is still generally recommended [325]. The use of urine transport media in vacuum-filled tubes with preservative (i.e. boric acid) or refrigeration immediately after collection decreases the proliferation of contaminating organisms and increases the likelihood of interpretable results. Urine samples that are delayed in processing and held at room temperature for extended periods of time (> 4 hours) are likely to demonstrate moderate to large increases in colony counts that are misleading and, in the majority of cases, are not recommended for culture [325]. Straight or “in-and-out” catheterization of a properly prepared patient usually provides a less contaminated specimen for culture. It should be noted that while urine samples collected via a midstream void or straight catheterization are preferred to optimize

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urine culture performance, some diagnostic testing, specifically NAAT for STI, requires a first voided sample for optimal performance.

Specimens from urinary catheters in place for more than a few hours frequently contain colonizing microbiota 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 that has been in place for <48 hours. Cultures of Foley catheter tips and urine from the bag of a catheterized patient are of no clinical value and should be rejected. Collection of specimens from urinary diversions such as ileal loops and nephrostomies is also discouraged because of the propensity of these locations to be chronically colonized. Bagged urine collections, primarily used for pediatric patients, are also of questionable value and should be strongly discouraged in favor of straight catheterization.

In patients with urinary stents, kidney stones, urologic abnormalities, or recurrent UTIs, infection may be caused by more than one organism (i.e. polymicrobial) or opportunistic pathogens, such as coagulase-negative staphylococci. For patients with stents and kidney stones, culture of the removed stent or stone is appropriate and may provide additional information if the patient develops urosepsis subsequent to removal; however, results should be interpreted in the context of the pathogens identified, the method of device or stone removal, and other laboratory data, including urine culture. For patients with chronic or recurrent UTIs urine culture conditions and interpretive and reporting criteria (i.e. lower reporting thresholds) may require modifications to identify organisms that are not typically recovered and reported using standard urine culture (SUC) approaches. Although it is clear that expanded quantitative urine culture (EQUC) methods, when compared to SUC, can recover additional potential urinary tract pathogens in lower quantities, their clinical relevance has not been fully elucidated and data do not support routine use of these methods for all patient populations [326]. 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, and laboratories must be aware of these requests prior to culture.

Specimens obtained by more invasive means, such as cystoscope or suprapubic aspirations should be clearly identified and are routinely cultured to allow for recovery of bacteria in concentrations as low as 1000 CFU/ml, as part of standard procedures. Identification of a single potential pathogen in numbers as low as 200 CFU/mL may be significant in some cases, but if the clinician is interested in recovery of bacteria in concentrations less than 1000 CFU/ml, the workup must be discussed in advance with the laboratory to achieve this lower level of detection.

While not without some exceptions, in febrile infants and young children (2–24 months) an abnormal urinalysis and a colony count of >50 000 CFU/mL of a single organism obtained by catheterization is considered diagnostic [327]. Some sites will work up any number of CFUs from suprapubic aspirates of pediatric patients. More recent evidence would suggest that $\geq 10^4$ CFU/mL

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and a reliable detection of pyuria would pick up an additional significant proportion of children with true UTI [328].

Recovery of yeast, usually *Candida* spp, even in high CFU/mL is not infrequent from patients who do not actually have UTI, thus interpretation of quantitation in cultures yielding yeast is not as standardized as that for bacterial pathogens. Yeast in urine may rarely indicate systemic infection, and clinical correlation with additional diagnostic testing is recommended (blood cultures, for example), specifically in groups at high risk for disseminated infection (i.e., patients with neutropenia or undergoing a urologic procedure) [329]. 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 collection containers, processing and media are employed. Identification of adenovirus in cases of cystitis is typically performed using nucleic acid amplification testing (NAAT), which is primarily available at reference laboratories. Polyoma BK virus nephropathy is best diagnosed by quantitative molecular determination of circulating virus in blood rather than detection of virus in urine. Testing for BK virus is routinely performed in academic medical center laboratories, larger medical center laboratories, or reference laboratories and FDA approved methods are also available.

While there are no current rapid molecular-based FDA approved technologies available for diagnosis of urinary tract infection, several applications of next generation sequencing direct on urine are in development or are commercially available. This type of testing strategy is not appropriate for all patient populations and clinical presentations, and there is concern that utilization of this technology in the evaluation of lower urinary tract symptoms may lead to overtreatment with antibiotics. Studies evaluating utilization of these technologies are scant and more evidence is needed before these technologies become widely adopted [330, 331].

Prostatitis

Acute bacterial prostatitis is defined by clinical signs and physical findings combined with urine positive for microorganisms. Prostate massage is of limited diagnostic value in acute prostatitis and increases the risk of bacteremia, and thus should be avoided [332-334]. The diagnosis of chronic prostatitis is much more problematic, and the percentage of cases in which a positive culture is obtained is much lower. It is important to carefully develop the differential diagnosis, as symptoms may overlap with other conditions including chronic pelvic pain syndrome, sexually transmitted infection, prostate cancer, urethral stricture, and others [335]. The traditional Meares-Stamey four-glass for chronic prostatitis is considered the diagnostic standard; however, it is cumbersome and rarely performed or ordered. The diagnostic principle is based on lower leukocyte and bacterial counts in voided bladder (VB) urine specimens from the urethra (VB1) and bladder (VB2), compared with counts in post-prostatic massage voided urine (VB3) or expressed prostatic secretions (EPS). A two-glass modification, comparing pre- with post-prostatic massage urine specimens, may also be used [336]. Table 39 summarizes the approach to laboratory diagnosis of prostatitis.

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Epididymitis and Orchitis

The evaluation and diagnosis of acute epididymo-orchitis overlaps with acute epididymitis. Epididymitis in sexually active post pubertal boys and men under 35 years of age is most frequently associated with the sexually transmitted organisms *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Epididymitis in pre pubertal boys and adolescents may be due to retrograde ascent from the urethra. Evaluation for underlying genitourinary tract abnormalities is warranted, especially in cases of recurrent epididymitis in pre pubertal boys. In men over 35 years of age, *E. coli* (and other Enterobacterales), *Pseudomonas* spp, and Gram-positive organisms that cause UTI and prostatitis may also cause invasive infections of the epididymis and testis. Urinalysis, urine culture, and a urine sample for NAAT for sexually transmitted infections are recommended. NAAT testing, which is the most sensitive method to detect organisms associated with GC and CT, is widely available commercially, but specific collection devices and transport containers are typically needed [204]. Surgically obtained tissue may be cultured for bacterial pathogens, and AST will be performed depending on the organisms isolated. *Brucella* spp., *Ureaplasma* spp., *Mycoplasma genitalium*, *Trichomonas vaginalis*, fungal (including blastomycosis, histoplasmosis and coccidioidomycosis) and mycobacterial disease (primarily due to *Mycobacterium tuberculosis*) are less common in both prostatitis and epididymo-orchitis, and laboratory diagnosis requires communication from the clinician to the laboratory to ensure proper transport medium selection, processing, and diagnostic methods. Additional diagnostic testing and follow up is recommended if symptoms do not improve within 48-72 hours of initiation of antibiotic therapy.

With the exception of mumps, isolated orchitis without epididymitis is uncommon in adults. Diagnostic testing includes the detection of mumps virus RNA by RT-PCR, IgM serology for mumps antibodies, or acute and convalescent IgG serology. Other viral causes of orchitis, primarily in children and adolescents, are Coxsackie virus, Rubella virus, Epstein-Barr virus and Varicella-Zoster virus. Laboratory diagnosis requires communication from the clinician to the laboratory to ensure proper transport medium selection, and processing, and diagnostic methods. Table 40 summarizes the approaches to specimen management for cases of epididymitis and orchitis.

Table 38. Laboratory Diagnosis of Cystitis and Pyelonephritis

Etiologic Agents	Diagnostic Procedures	Optimal Specimens	Transport Issues
Gram-negative Bacteria			
Enterobacterales: Includes *Escherichia coli, Klebsiella* spp, *Proteus* spp, *others*	Urine culture Gram stain (optional, low sensitivity)	Mid-stream voided or straight catheter urine	Sterile leakproof container; refrigerate (4°C) or use urine transport tube with preservative unless delivery to

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Pseudomonas spp, other non-fermenting gram-negative bacilli			laboratory ≤1 h is certain.
Gram-positive Bacteria/			
Enterococcus spp. *Staphylococcus aureus* *Staphylococcus saprophyticus* *Corynebacterium ureolyticum* *Streptococcus agalactiae (*Group B streptococci*)*	Urine culture Gram stain (optional, low sensitivity)	Mid-stream voided or straight catheter urine	Sterile leakproof container; refrigerate (4□C) or use urine transport tube with preservative unless delivery to laboratory ≤1 h is certain.
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	First void urine	Prefer >20 mL urine, refrigerate (4□C) during transport
Virus			
Adenovirus	NAAT[1]	Midstream voided urine	Sterile container
BK Polyoma virus	Quantitative NAAT from urine, plasma, or serum	Blood Serum	EDTA or Citrate blood collection tube, RT Clot tube, RT

[1]No FDA-cleared NAAT tests available

Table 39. Laboratory Diagnosis of Prostatitis

Etiologic Agents	Diagnostic Procedures	Optimal Specimens	Transport Issues
Acute Bacterial Prostatitis			
E. coli, other Enterobacterales, *Pseudomonas* spp *Staphylococcus aureus* *Enterococcus* Group B streptococci	Urine Culture	Midstream voided urine (without expressed prostate secretions)	Sterile leakproof container; refrigerate (4□C) or use urine transport tube with preservative unless delivery to

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			laboratory ≤1 h is certain.
Chronic Bacterial Prostatitis			
Pathogens similar to acute bacterial disease	Urine culture Gram stain (optional, low sensitivity)	Midstream voided urine and expressed prostate secretions, seminal fluid	Sterile leakproof container; refrigerate (4°C) if delayed transport
Fungus			
Blastomyces dermatitidis *Coccidioides immitis/posadasii* *Histoplasma capsulatum*	Fungal culture	Prostate biopsy	Sterile container refrigerate (4°C) if delayed transport
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	First void urine, prostate biopsy	Prefer >20 mL urine, sterile container, refrigerate (4°C) during transport; sterile container refrigerate (4°C) if delayed transport

Table 40. Laboratory Diagnosis of Epididymitis and Orchitis

Etiologic Agents	Diagnostic Procedures	Optimal Specimens	Transport Issues
Bacteria			
Chlamydia trachomatis *Neisseria gonorrhoeae* *Mycoplasma genitalium* *Trichomonas vaginalis*	NAAT	Urethral swab or first void urine for NAAT	Specific collection system for each NAAT system
Enterobacterales, *Staphylococcus aureus*	Urine culture Tissue or biopsy culture	Urine Tissue aspirate or biopsy	Sterile container, refrigerate (4°C) if delayed in transport.
Virus			

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Mumps Coxsackie Rubella EBV VZV	Serology and/or NAAT	Contact laboratory for recommendation	Contact laboratory for recommendation
Fungus			
Blastomyces dermatitidis, Coccidioides immitis/posadasii, Histoplasma capsulatum/duboisii	Fungal culture	Tissue aspirate or biopsy	Closed sterile container, refrigerate (4°C) if delay.
Mycobacteria			
Mycobacterium tuberculosis	Mycobacterial culture	First void urine Tissue aspirate or biopsy	Prefer >20 mL urine, sterile container, refrigerate (4°C) during transport; sterile container, refrigerate (4°C) if delayed in transport.

XII. GENITAL INFECTIONS

In 2019, the rates of reportable sexually transmitted infections (STIs) in the US reached an all-time high with about 1 in 5 Americans having an STI. This rate is likely higher, given not all STIs are reportable. Because routine screening is inadequately implemented and risk of long-term morbidity is increased, a paradigm shift in the national response strategy has been proposed, including leveraging healthcare systems and clinicians not traditionally involved in STI services and new biomedical tools [337]. In addition, diagnostics strategies implemented during the pandemic are now surging forward to accelerate STI detection. These include a broader range of deployable diagnostics in a variety of settings, innovative approaches to expand testing to address access to care and optimize timeliness of treatment, and instrument-free diagnostics. The standards for infectious disease diagnostic tests have been updated by the World Health Organization (WHO) with the criteria that has the acronym: REASSURED (Real-time connectivity, Ease of specimen collection, Affordable, Sensitive, Specific, User-friendly, Rapid and Robust) as well as ease of use for personnel performing the testing. WHO Target Product Profiles (TPP) clarify that focus is global, for low- and middle-income countries (LMIC) as well as higher-income countries [338].

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In this section, both point of care and laboratory-based tests to identify the microbiological etiology of genital infections are described. Data recently published from new diagnostic test performance and clinical utility data may contrast with other guidelines. In tables, many conventional tests have been removed compared to previous versions because they are no longer the current standard of care (SOC) and/or current data suggest that use for diagnosis is inadequate. Readers are referred to reference laboratories for specialized testing needs. Infections are categorized as follows: mucocutaneous and cutaneous genital lesions, vaginitis and vaginosis, urethritis and cervicitis, and infections of the female pelvis, including endometritis, pelvic inflammatory disease (PID) and post-partum infection. Testing in special populations, such as pregnant patients, children, men who have sex with men (MSM) and transgender persons 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 or syndromic assessment alone has been shown in multiple studies to be neither sensitive nor specific for several entities including *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), bacterial vaginitis/vaginosis (BV), Trichomonas, Candida, and genital lesions [339-341]. In, addition, the increased use of empiric treatment has raised significant concerns about both antimicrobial resistance and alterations in the microbiome [339, 342, 343]. Thus, diagnostic testing is recommended for the following reasons: directed appropriate treatment rather than empiric antimicrobials for eradication, reduction of transmission as well as symptomatic relief, increased therapeutic compliance by the patient, and patient more likely to comply with partner notification [204]. However, providers should recognize that despite diagnostic testing, 25-40% of the causes of genital infections or symptoms may not be specifically identified, due to several reasons including; lack of an available diagnostic test or an unrecognized medical entity, as well as an incomplete sexual health history that results in missed opportunities for appropriate testing [344]. 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 (STIs) are likely to have been re-infected by their sexual partner(s) [345]. Thus referral for partners for specific testing and/or directed treatment is essential to prevent re-infection and is especially important for patients who may be pregnant or HIV positive [204]. 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 many STIs including CT, GC, syphilis, chancroid, mpox and human immunodeficiency virus (HIV), acute hepatitis C virus (HCV), and hepatitis B virus (HBV), require reporting in accordance with state and local statutory requirements by the laboratory and/or the provider. Reporting of additional STIs varies by state [346].

Key points for the laboratory diagnosis of genital infections:

- o HIV testing is recommended for all persons with unknown status seeking STI evaluation

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- Syphilis screening in pregnant patients may be required by state law to be performed more than once during the pregnancy
- Appropriate testing in transgender care requires clarification of anatomy at the time of exam
- Managing appropriate and directed therapy is an important strategy in antimicrobial stewardship and resistance development in STIs
- Clinicians other than STI and ID specialists are needed to support the goals of the STI national plan
- Some NAAT technologies require a specific manufacturer collection/transport device for STIs, or results could be compromised and specimens rejected. Use devices recommended by the laboratory
- Multiplex molecular assays for detection of several organisms associated with bacterial vaginosis are more specific and sensitive than syndromic assessment alone (Amsel's), Nugent Gram stain or hybridization probe testing that only includes *G. vaginalis*
- In patients being tested for vaginitis, adding testing for CT/ NG identifies approx. 25% more infections in high-risk populations
- Aerobic vaginitis is a unique pathologic entity different from bacterial vaginosis that may require Gram stain and vaginal culture. Contact laboratory on how best to submit specimen. Often labs will classify this specimen as wound to provide the appropriate work-up
- Trichomonas is now the most common STI in certain populations but remains a non-reportable STI to public health
- Females with identified risk should also have extra-genital sites tested for CT/NG
- Cervical cancer screening recommendations promote three accepted pathways depending on age: cytology only, co-testing, and primary HPV screening only
- Prenatal screening interval for GBS has moved closer to gestation, from 35-37 weeks to 36 0/7 to 37 6/7. Screening both rectal and vaginal sites and using nutrient broth enrichment before testing offers greatest sensitivity.

Genital lesions

Genital lesions or Genital Ulcer Disease (GUD) may have multiple simultaneous infectious etiologies that make them a challenge to diagnose and treat properly. In addition, several etiologies also present with oral ulcers. Like many other STIs, syndromic assessment yields poor sensitivity and specificity for correct diagnosis [341]. FDA-cleared, rapid diagnostic molecular tests for pathogens associated with genital lesions (HSV1/2, mpox, syphilis) including HIV and able to be used in pregnant patients are needed for appropriate management. Because many genital lesions exhibit an inflamed epithelium that enhances the transmission of HIV, screening with an EIA (enzyme-immunoassay) HIV antibody test is recommended in all patients presenting with genital, anal or perianal lesions. In addition to diagnostic tests for syphilis, genital herpes and mpox should

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be considered based on sexual history, symptoms and complete physical exam. *H. ducreyi* or chancroid, Lymphgranuloma venereum (LGV), and granuloma inguinale (donovanosis) are rare in the US and specialized testing procedures are required [204].

Rapid POC HIV or home tests can enable a preliminary diagnosis of HIV infection, but the majority of rapid antibody assays become reactive 3 months after exposure compared to laboratory-based assays and thus can yield negative results in people recently infected. All FDA-cleared laboratory-based Human Immunodeficiency Virus Types 1 and 2 (HIV-1/2) diagnostics are Ag/Ab combination immunoassays and highly sensitive and specific, including for known subtypes of HIV-1, HIV-2 and uncommon variants of HIV-1 (e.g., group O and group N). Whenever acute HIV infection is suspected and initial CDC algorithm testing is negative or indeterminate, testing for HIV RNA is recommended [347].

Table 41 shows the diagnostic tests for identifying the etiology of the most common genital lesions. For suspected cases of HSV genital lesions, NAATs are standard of care (SOC). Diagnosis with direct fluorescent antibody (DFA) or cell culture are performed more commonly through reference laboratories and may require special transport conditions. Consultation with the laboratory is recommended. Clinicians need to recognize that HSV-1 is now commonly seen as a genital pathogen, especially in young women and MSM and that atypical VZV presentations occur, especially in children [204]. Most laboratories performing NAATs usually offer HSV 1 and 2 as well as VZV from the same specimen. Typically, a generic lesion swab specimen is acceptable, that includes mucocutaneous, vesicular or even crusted lesions, and collected in Universal Transport Medium (UTM) which can be transported at room temperature. Several FDA-cleared NAATS and Laboratory Developed Tests (LDTs) exist. Consultation with the laboratory before specimen collection is appropriate as there may be limitations as to specimen source able to be tested and/or patient age depending on the test being used. NAATS are the preferred diagnostic method because they provide typing to specify HSV type 1 or 2, are the most sensitive, especially where suboptimal collection or non-ulcerative or vesicular lesions may be present [341, 348-350]. While DFA allows assessment of specimen adequacy and can performed within a few hours if performed on site and is more specific than a Tzanck preparation, it is labor-intensive and less sensitive than NAAT. All specimens if positive 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 performed, usually through a reference laboratory. Point of care tests for HSV-2 may yield false positive results in patient populations with a low likelihood of HSV infection and in primary lesions that are due to HSV-1 and false negative results when HSV-2 is in early stages of infection. Because both HSV-1 and 2 occur as genital infections, antibody testing is rarely indicated [204].

In children presenting with genital lesions, providers should not assume HSV as the only etiology and should consider potential atypical presentation of herpes zoster virus (VZV) or even mpox depending on clinical and family history or exposure. FDA-cleared VZV NAATs are limited but

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several reference laboratories offer these tests or LDT NAATS. Pregnant patients with a history of genital herpes should be assessed for active lesions at the time of delivery but screening with molecular assays is not appropriate.

Syphilis has seen an unprecedented surge, including significantly increased rates in congenital syphilis. Screening is recommended in many settings, as it is a treatable disease. In the United States, rapid diagnostic tests with good performance parameters for diagnosing primary syphilis are lacking [351, 352]. In addition, darkfield smear exams and NAATS for detecting *T. pallidum* directly from lesion exudate or tissue, while recommended by CDC, are not readily available in clinical laboratories and lack sensitivity in early disease. Typically, testing for syphilis is performed by serology and requires two tests. Traditional testing has consisted of initial screening with an inexpensive non-treponemal test (i.e., Rapid plasma reagin, [RPR]), then retesting reactive specimens with a more specific, and more expensive, treponemal test (i.e. *T. pallidum* particle agglutination [TP-PA]). If a non-treponemal test is being used as the screening test, confirmation is needed, as a high percentage of false positive results occur in many medical conditions unrelated to syphilis including other infections (e.g., HIV), autoimmune conditions, vaccinations, (including Covid 19 vaccinations), injection drug use, pregnancy, and older age. When both test results are reactive, they indicate present or past infection. Now more commonly, high-volume clinical laboratories have reversed the testing sequence and begin the testing algorithm first with a specific treponemal test, such as an EIA or chemiluminescence immunoassay (CIA), and: then retesting reactive results with a non-treponemal test, such as RPR to confirm diagnosis. Both of these screening algorithms have shown equivalent performance for detection of active infections. Screening with a treponemal test can identify persons previously positive, treated and/or partially treated for syphilis as well as yield false positives in patients with low likelihood of infection. If the follow-up confirmation test (RPR) is negative it requires the laboratory to perform a second treponemal test to guide management decisions (i.e., FTA-ABS). *T. pallidum* cannot be seen on Gram stain and cannot be cultured in the routine laboratory. There is one FDA-cleared rapid HIV-Syphilis test, CLIA waived, that uses fingerstick blood and meant to be used for point-of-care (POC). The DPP® HIV-Syphilis System, (Chembio Diagnostics, Inc.), is a single use, rapid, qualitative, multiplex, immunoassay for the detection of antibodies (not antigens) to HIV Types 1 and 2 (HIV-1/2), and/or *Treponema pallidum* bacteria. ,Results are presumptive and used with routine laboratory multi-test algorithms designed to diagnose HIV and syphilis. A review of current POC syphilis tests globally, showed good sensitivity (85%) and high specificity 98% [352]. Clinical utility studies are needed in POC sites where syphilis management could have high impact.

Monkeypox virus is an *Orthopoxvirus* that has historically caused sporadic human cases linked to animal contact in Africa with rare human-to-human spread. Two major clades have been identified: clade I (formerly Congo Basin) and clade II (formerly West African) with clade I known to cause more severe disease. Human to human transmission primarily occurs through close personal contact with direct exposure to lesions or body fluids. Symptoms begin about 5-13 days

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after exposure and include fevers, headache, sore throat, back pain, muscle aches, and fatigue. The rash usually starts a day or two later at the inoculation site. The rash progresses from macules to pustules and in about 2 weeks, the lesions crust and fall off. Lesions are very painful. In May of 2022, Monkeypox virus clade II reemerged and spread globally very quickly with about 30,286 cases reported in the US [353]. Cases primarily occurred in men who have sex with men (MSM), were different from other outbreaks being specifically clade II, with presentations that were atypical compared to other outbreaks (e.g.no prodrome, atypical rash distribution on the body, no visible lesions). Primed from the COVID-19 pandemic, the immediate response was to identify those at risk, ramp up diagnostic testing, vaccination and treatment, and determine isolation procedures to reduce spread. Public Health Laboratories in the CDC Laboratory Response Network (LRN) were able to deploy the CDC developed 510k approved non-variola *Orthopoxvirus* test; the test was later made available to five large commercial labs. The US FDA Emergency Use Authorization (EUA) pathway for diagnostic tests was opened in September 2022. In addition, the NIH RADx (Rapid Acceleration of Diagnostics) and the Independent Test Assessment Program (ITAP) began accepting applications from test developers of high throughput and POC tests for accelerated review [354]. The Select Agent program eased reporting requirements for positives during this clade II 2022 outbreak. Specimens most commonly used for testing, and the specimen type on-label for all EUA tests, are lesion swabs in VTM or UTM. Diagnostic laboratory personnel can work in a BSL-2 laboratory facility following standard and special practices, safety equipment, and facility specifications. The first mpox CLIA-waived tests using lesion swabs, received EUA in February and March 2023 (Xpert®Mpox and Cue mpox molecular test, respectively Clinicians should check with laboratories for specific specimen requirements, utilize appropriate PPE during the patient assessment to reduce exposure risk, and educate patients on required isolation procedures (https://www.cdc.gov/poxvirus/monkeypox/index.html). CDC has the original non-variola OPX test that has 510k and several diagnostic tests have EUA, including LDTs. Links for both options are included here. https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices#molecular;

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/monkeypox-mpox-and-medical-devices#Laboratories

Chancroid, caused by the gram-negative organism *Haemophilus ducreyi,* lymphogranuloma venereum (LGV), caused by *C. trachomatis* serovars L1, L2 or L3 and Granuloma inguinale (donovanosis) caused by the intracellular gram-negative bacterium *Klebsiella granulomatis* cause genital ulcers uncommon in the US and are typically diagnosed by clinical presentation, identification of high risk-factors and exclusion of the more common etiologies of genital lesions, such as syphilis, HSV, and mpox. Chancroid may be identified by Gram stain and specialized culture medium to isolate colonies, but not recommended to be performed unless by a laboratory experienced in this testing. Current NAATS for CT will detect serovars associated with LGV but are not able to differentiate specific serovars. No NAATS are cleared for genital ulcer sites. Rectal

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swabs are cleared for many NAATS, and patients with proctitis are recommended for testing [204, 355]. Serology and MIF can be found through reference laboratories. MIF titers ≥ 256 with appropriate clinical presentation suggests LGV. Scabies is a skin infestation which causes pruritis, by the mite *Sarcoptes scabiei.* Scabies in adults is often associated with sexual exposure, but in children it is not. Diagnosis can be made by identifying burrows, mites, eggs or feces from the affected area in the clinical setting, with the aid of a videoscope or magnifying lens which are non-invasive. While skin scrapings are often used, this is painful and diagnosis often delayed. Pediculosis pubis, or pubic lice, is caused by the parasite *Phithirus pubis* and typically is transmitted through sexual exposure. Patients seek medical attention because lice or nits are noticed on pubic hair. Diagnosis is made by visual inspection of lice by the clinician or by microscopy from material submitted to the laboratory by scrapings into sterile container [204].

Human papilloma virus (hpv)

High risk HPV (hrHPV) types are responsible for most HPV-related cancers and include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Two of these, HPV16 and HPV18, are responsible for most cancers. HPV guidelines and recommendations address what is considered the optimal prevention strategies that would identify those HPV-related abnormalities likely to progress to invasive cancers while avoiding destructive treatment of abnormalities not destined to become cancerous. HPV testing refers to molecular HPV assays that have been analytically and clinically validated for screening of cervical cancer and verified pre-cancer cervical intraepithelial neoplasia 2+ (CIN2+) by the U.S. Food and Drug Administration (FDA). Updated cervical cancer screening recommendations were reported by the U.S. Preventive Services Task Force (USPSTF) in 2018 and endorsed by The American College of Obstetricians and Gynecologists (ACOG), American Society of Colposcopy and Cervical Pathology (ASCCP) and Society of Gynecologic Oncology (SGO) in April 2021 [356]. There are three screening options:

1. hrHPV testing alone every 5 years initiated at age 30
2. hrHPV testing plus cytology every 5 years (co-testing) Initiated at age 25
    - Five HPV tests are FDA-approved for co-testing
3. Cytology alone every 3 years initiated at age 21-29

The acronym hrHPV designates high risk HPV testing that detects genotypes that have high risk for cervical cancer and does not include low risk HPV types. However, no cervical cancer screening tests detect low risk types or are recommended. Thus, hrHPV is somewhat redundant. Primary HPV screening is a streamlined term promoted by the American Cancer Society (ACS).

ACS recommendations differ slightly by preferred screening method and age of initiation. The 2022 guidelines are deemed “transitional” [357]:

Primary HPV screening

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1. Only option
2. Testing initiated at age 25, instead of at 21

However, because there are currently only 2 FDA-cleared primary HPV screening platforms in the US, the ACS recognizes the limitation of their recommendation, and thus the inability to uniformly incorporate primary HPV screening nationwide. ACS agrees to the testing options listed above by ACOG, ASCCP and AGO if primary screening is not available, with the exception of initiating all testing at age 25, not age 21. The next ACS guidelines will not include cytology as a component of future screening options as data and modeling has shown that cytology is less sensitive and specific for determining cancer risk and co-testing is less efficient. Having a single screening guideline recommendation should allow for easier and consistent implementation, as currently a large number of providers do not follow current guidelines (e.g., still performing annual cervical exams). Providers should be aware that HPV testing can be performed in different laboratories including cytology, and/or molecular laboratories.

Common to all guidelines [356, 357].

- All recommendations apply to those patients who are *average risk*. Average risk is defined as any person with a cervix who does not have any signs or symptoms of cervical cancer regardless of their sexual history or HPV vaccination status.
- The recommendations do not apply to individuals at *increased risk* for cervical cancer due to solid organ or stem cell transplantation, human immunodeficiency virus infection or immunosuppression from other causes, or in utero exposure to diethylstilbestrol.
- No screening guidelines recommend yearly exams for cervical cancer. Education is needed to change this practice.
- Screening should not begin before the age 21, applies to any asymptomatic individuals with a cervix regardless of their sexual history or human papillomavirus (HPV) vaccination status, those who have undergone hysterectomy with cervix still remaining, and transgender persons who retain their cervix.
- The ACS recommends that individuals aged >65 years who have no history of cervical intraepithelial neoplasia grade 2 or more severe disease within the past 25 years, and who have documented adequate negative screening in the prior 10 years, discontinue all cervical cancer screening. Ending of screening needs to be reviewed as data accumulates.
- In patients with hysterectomy and removal of cervix – no screening is necessary unless prior history for high-grade cervical precancerous lesions or cervical cancer

Follow-up testing after co-testing with abnormal cytology and/or positive hrHPV is complicated and readers are referred to the ASCCP guidelines for management decisions and the free teaching modules [358].

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Significant pending issues for HPV screening include: education to clinicians and laboratorians on guideline recommendations and consistency in implementation moving forward, the use of self-collected vaginal specimens which are not currently FDA-cleared in the U.S., but have shown promise in difficult to reach patients, acceptability to women and not subject to the issues of inadequate specimen sample seen for cytology analysis, consensus/data on when discontinuation of routine screening occurs, and effect of increased uptake of HPV vaccination and impact for future screening risk in those vaccinated, as a decrease in HPV infections is starting to emerge.

HPV – genital warts

Genital warts are a sexually transmitted infection caused by certain types of human papillomavirus (HPV). The main clinical manifestation of genital warts is benign hyperplasia of the skin and mucous membrane in the genitalia, anus and perineum. A variety of HPV types can cause genital warts, but HPV 6 and 11 together account for about 90% of all cases. Most diagnoses are made by visual inspection and/or biopsy. Typing is not commonly performed.

Prevention of HPV and cervical cancer starts with vaccination. CDC now recommends two doses starting at ages 11-12, 6-12 months apart, available for both boys and girls. Vaccination can start as early as age 9. Those who start later than age 15-26 need 3 doses. There are currently 3 HPV vaccines licensed by the FDA, but since late 2016, only Gardasil-9 (9vHPV) is distributed in the United States. This vaccine protects against nine HPV types including types 6, 11, 16, 18, 31, 33, 45, 52, and 58 [359].

Vaginitis/vaginosis

Significant clinical utility and outcome data has emerged for diagnostics used for detection of the conditions related to vaginosis/vaginitis. These conditions result in millions of visits by women each year, often repeatedly because of misdiagnosis and inappropriate treatment [360]. The diagnoses of bacterial vaginosis (BV), a condition caused by an overgrowth of altered normal vaginal microbiota, and vaginitis caused by fungal organisms (vulvovaginal candidiasis [VVC]) or *Trichomonas vaginalis* (TV), are often considered clinically and diagnostically as a group because of their overlapping signs and symptoms and together account for about 90% of vaginitis (Table 42). The mode of transmission and/or acquisition is not necessarily that of an STI for VVC, but may be for BV and is for TV. Inflammation in vaginitis puts patients at increased risk for STI acquisition, including HIV, as well as complications after gynecologic surgery and pregnancy. Several studies identify the high percentage of co-infections among the vaginitis entities (TV, VVC, BV) as well as infections with CT and GC, questioning the need for routine screening for all these entities in women with vaginitis [204, 343, 361-363].

Many guidelines still recommend use of diagnostic tests for BV that are poor in sensitivity and specificity citing lower cost and ability to provide a rapid diagnosis [204, 364]. However, these recommended tests are not commonly available in laboratories or rapid and often not diagnostically valid. In fact, Nugent Gram stain yields indeterminate results 25-30% with initial

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smear interpretation and is a high complexity test requiring significant training. *G. vaginalis* cultures are not recommended as 55% of women without BV harbor this organism and reporting this organism alone for BV determination results in poor specificity and overtreatment. Likewise, the Affirm VPIII (Becton-Dickinson), which detects only *G. vaginalis* as the determinant for BV, like culture, has very poor specificity [340, 365]. While sensitivity for VVC of the Affirm VPIII is adequate compared to culture, that for TV ranges from 63 to 100% compared to the SOC NAAT [361, 365].

Point-of-care tests (POCTs) that can be performed from a vaginal discharge specimen while the patient is in the healthcare setting to meet Amsel's criteria for BV, e.g., visualization of thin whitish discharge, vaginal pH strip performed at the POC, potassium hydroxide/whiff test, and wet mount microscopy, have been documented to be rarely performed (only 1 in 5 providers) [340, 343, 366-368]. As well, multiple publications document the unacceptable poor performance of these POCTs by providers when compared to laboratory-based diagnostics, lacking both sensitivity and specificity for making an accurate diagnosis. The overall result with inadequately performed POCTs is that women receive syndromic management and empiric antimicrobials, with nearly 50% of women being incorrectly treated, including both over treatment and missed treatment [340, 342, 343, 369]. Empiric antibiotic use results in creating alterations in the vaginal microbiome, increases resistance to fluconazole for yeast and metronidazole for TV, an overall decrease in patient satisfaction, continued symptoms, and repeat visits with increased costs [343, 368].

For VVC and TV, whether at POC or in the laboratory, the presence of pseudohyphae in saline wet mount with KOH or motile trichomonads visualized in wet mount, 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 relative to culture and NAAT, respectively [366]. While culture for yeast is SOC, it takes several days resulting in many women incorrectly treated with OTC medications unnecessarily [342].

Several NAATs exist for TV which is SOC. It should be noted that recent publications utilizing NAATs highlight the prevalence of *Trichomonas* as equal to or greater than CT and GC in certain patient populations and point to a growing trend toward screening for TV, CT and GC simultaneously, especially in younger women [362, 370, 371]. One CLIA-waived rapid antigen test, OSOM® Trichomonas, performs fairly well in symptomatic patients (90%). It is approved for use only in patients >= 18 years of age.

Multiplex naats for vaginitis

Like the initial investigations of NAATs compared to culture for CT and NG, the SOC reference methods for vaginitis limit the validity of interpretation of the new multiplex vaginal panels. Basically, the vaginal Gram stain (Nugent) and Amsel's criteria do not align with each other on either sensitivity or specificity [369]. Currently, there are three FDA-cleared microbiome-based

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multiplex vaginal NAATs, BD Max™ Vaginal Panel (Becton Dickinson) (for use in women ≥18yo), Aptima® BV and CV/TV (Hologic) (both approved for use in ≥14 years of age), and the Xpert® Xpress Multiplex vaginal panel (MVP) test (Cepheid) (approved for use in women ≥18yo).

Several commercial labs offer testing for vaginitis, often requiring a specific swab. Providers need to be aware that targets may vary depending on assay platform used. Tests offered vary from FDA-cleared platforms to lab developed (LDTs).. FDA-cleared tests, have been validated in several publications [365, 372-376]. All tests are for use in women with symptoms consistent with vaginitis/vaginosis with either a single self-collected or clinician-collected vaginal swab specimen. Importantly, these multiplex tests are not intended for screening asymptomatic patients. They are also not to be used for prognostic purposes or to be used as test of cure. In general, multiplex tests have provided more accurate diagnoses for causes of vaginitis, consistently demonstrating higher sensitivity and negative predictive value than clinician diagnosis or POCTs. In addition, a statistically higher overall percent agreement with each of the reference methods than SOC POCTs performed on site demonstrated statistically higher sensitivity for detecting co-infections, most commonly, BV and VVC.

BV targets and interpretation algorithms differ for each product, but all use multiple vaginal microbiota species for determination of a positive result, making the tests specific for BV. Candida species are identified in groups relative to likelihood of fluconazole susceptibility (fluconazole susceptible, e.g., *C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis* versus fluconazole resistant e.g. *C. glabrata, C. krusei*). In addition, NAATs have been more accurate in identifying mixed and co-infections, both among vaginitis entities (BV, VVC, TV) as well as with CT and NG [362]. Outcome data from both prospective and retrospective review of claims data and studies shows that primary testing with NAATs results in fewer repeat visits, more directed therapy and less overall cost as the primary testing choice compared to current SOC POC, despite NAAT results compared were not available at the POC [368]. Overall, data suggests that the need for consistent, more accurate diagnosis and directed treatment is needed [343]. A transition to accurate diagnostic testing for vaginitis by multiplex NAATs needs to be thoroughly addressed in future guidelines.

Aerobic vaginitis

Aerobic vaginitis (AV) is an entity which is characterized by abnormal vaginal microbiota consisting of aerobic enteric commensals or pathogens, including Group B *Streptococcus* (*S. agalactiae*), *Enterococcus faecalis, Escherichia coli*, and *S. aureus*, and variable levels of vaginal inflammation eliciting a specific host-response, including infiltrating leukocytes and parabasal cells. The most severe form is desquamative inflammatory vaginitis, that often is found with vaginal atrophy. BV and AV share some similar characteristics, such as decreased lactobacilli and

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increased pH (typically higher in aerobic vaginitis (pH 6) but in aerobic vaginitis there is no positive whiff test, the vagina is red and edematous, often has small erosions or ulcerations, and the discharge is yellow-green. Dyspareunia is common. Co-infections with other pathogens exist, e.g., TV, as well as sequelae related to pregnancy complications, including ascending chorioamnionitis, premature rupture of membranes (PROM), and preterm delivery. AV has been observed in 8–11% of pregnant women and in 5–24% of women reporting vaginal complaints. The appropriate diagnosis and distinction between AV and BV is crucial as their treatments are very different, and metronidazole is inappropriate for BV. Diagnosis is made by reviewing vaginal discharge and a Gram stain or phase contrast microscopy visualizing enteric bacilli and gram-positive cocci, lack of lactobacilli, and presence of leucocytes and parabasal or immature epithelial cells. Newer assessments, including microbiome profiles by whole genome sequencing, instead of a pre-set menu, may allow better assessment of altered microbiota and personalized risk assessment and provide additional data in the future to help address this continuing changing field.

Cervicitis/urethritis

CT, NG and TV are the most identified pathogens and continue to steadily increase in the US and globally. Urethritis and cervicitis share common signs and symptoms and infectious etiologies in male and female patients. Table 43 outlines the diagnostic tests used to identify the pathogens common to both. Updates to diagnosis, include FDA-cleared extra-genital sites and point of care diagnostics that can be performed in the CLIA-waived setting. In addition, home-collection of specimens, while available in the past, is being used more in certain states and healthcare entities. These changes allow an increase in appropriate testing of sites of infection (self-collection), testing at the site of service to provide directed therapy, and potentially greater access to needed healthcare services. Home collection is not currently under the regulation of the FDA or an FDA-approved source for any STI testing, thus the sample integrity of home-collected specimens is the responsibility of the laboratory performing these tests. There is a gap in how home-collection and/or home testing should be regulated apart from the COVID-19 pandemic and EUA testing.

CT and NG continue to be the most common reportable STIs, especially in younger, atrisk populations. Because screening for CT and GC has reduced the repercussions related to infections and subsequent PID, the following guidelines have been presented by the US Preventative Task Force and recently updated CDC guidelines [204, 377]. A significant change is the addition of screening of extra-genital sites in women when sexual history identifies risk. Screening in men is not considered cost-effective, unless MSM with high-risk.

Annual CT screening

- Sexually active women age ≤ 25 years and those pregnant
- Older women with the following identified risk factors:
  - new sex partner
  - multiple partners

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- partner with an STI
- inconsistent condom use, not in a monogamous relationship
- previous or coexisting STI
- exchanging sex for money or drugs, incarcerated

Annual GC screening (consider local epidemiology and risk)

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

For laboratory diagnosis of CT and GC, NAATs are the preferred assays for detection because of increased sensitivity while retaining specificity in low prevalence populations (pregnant patients) and the ability to screen with a noninvasive urine specimen [204, 378]. Vaginal specimens for women (either provider or self-collected) and urine specimens for males are preferred specimen sources. In MSM, rectal and oropharyngeal testing is recommended, and several FDA-cleared NAATS exist for rectal and oropharyngeal sites. Conjunctival specimens often collected in neonates, with crusted eyelids, from mothers with no history of screening in pregnancy or high risk, are not currently FDA-cleared and require in-house validation. Providers need to confirm with the laboratory if these sources will be tested. In general, retesting patients with a follow-up test for CT or GC (test of cure) is not recommended unless special circumstances exist (pregnancy, continuing symptoms). However, patients who are at higher risk for STIs should be screened within three 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, especially in potential victims of assault; check with state guidelines. Appropriate providers or laboratories that perform testing in children should be consulted [379].

Recently, prevalence studies using NAATs have shown that *Trichomonas vaginalis* is as common as CT and more common that NG in certain clinical and geographic settings, with a uniquely high presence in older women and men and in incarcerated populations. In addition, the ulcerative nature of TV infection leads to sequelae like those of CT and NG, including perinatal complications as well as susceptibility to HIV and HSV acquisition and transmission. FDA-cleared NAATs allow testing from the same screening specimens used for CT and NG testing and/or from specimens collected for vaginitis with significantly improved sensitivity over wet mount or hybridization tests [361, 380].

There are currently two FDA-cleared CLIA-waved tests, the Binx Health io® CT/NG Assay (Boston, MA) for use with female vaginal specimens (aged 16and above) and male urine (aged 17 and above), and the Visby Medical™ Sexual Health Click for CT/NG/TV (San Jose, CA) (approved for ages ≥14 y/o), for use with female vaginal specimens. Both tests are cleared for screening or diagnostic testing, as well as clinician or self-collected specimens. Assays can be performed at any site operating under a CLIA Certificate of Waiver, Certificate of Compliance or Certificate of Accreditation, with results in about 30 min. Clinical performance estimates with testing performed by non-laboratory trained personnel for Visby, has shown 97-99% sensitivity

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and 97-99% specificity for CT, NG and TV, respectively compared to standard laboratory NAATs and infected patient status [381, 382]. Similarly, performance for Binx IO has shown sensitivity and specificity for CT of 96 and 99% and 92 and 99% for women and men, respectively and sensitivity and specificity for NG of 100 and 99% and 97.3 and 100% for women and men, respectively [383, 384]. Visby allows instrument-free testing and screening of the highest risk group, i.e., females, with optimal specimen type, a vaginal swab. The limitation to date has been inability to use other specimen types and a high initial indeterminate rate of >7%. Binx IO requires a small instrument and while it uses vaginal swabs for female and urine for male, it is not FDA-cleared for TV and extragenital sites, especially for MSM. For both systems, PPV in low prevalence settings will have to be assessed. However, these kinds of tests performed at the point of care, specimens sent from home or in smaller healthcare settings are important for STI detection and screening, as the trend in healthcare has shown a 6-fold increase in the reliance of urgent care visits within the US involving a diagnosis of unspecified STIs. Directed treatment in these settings is vital as these are often patients lost to follow-up [385].

M. genitalium is a recognized pathogen causing nongonococcal urethritis (NGU) and non-chlamydial NGU in males and likewise cervicitis and PID in females. 15-25% of infections may be due to this organism and resistance to first line agents, macrolide (i.e., azithromycin) or quinolone (i.e., moxifloxacin), is significant, especially in HIV positive males [394]. NAATs are the best option for detection of *M. genitalium*, due to lack of culture availability secondary to specialize growth needs and cross-reactivity with serologic tests with *Mycoplasma pneumoniae.* Currently, two NAATs for *M. genitalium* are FDA cleared (Hologic Aptima® M.Gen Assay, and Abbott's Alinity™ STI assay for use with urine and urethral, penile meatal, endocervical, and vaginal swab samples [386]. Routine screening is not recommended in asymptomatic men or women. Testing should be considered in persistent NGU or PID. Molecular tests for resistance markers to macrolides and fluoroquinolones are not commercially available in the US, but detection of mutations associated with these drugs are under evaluation. Culture or NAATs for *Ureaplasma* are not recommended because of the high prevalence of colonization in asymptomatic, sexually active people [387].

Much like HIV, pooling for testing is an emerging practice to reduce total testing and reduce costs for STI screening. Evaluations have included combining multiple recommended specimen sources, such as urine or urethral, rectal, and oropharyngeal from a single patient (e.g., MSM) and combining multiple different patient specimens for CT/NG testing. Both practices have shown valid sensitivity and specificity as well as cost savings [388, 389].

Infections of the Female Pelvis

Pelvic inflammatory disease (PID) refers to acute and subclinical infection of the upper genital tract in females, involving any or all of the uterus, fallopian tubes, and ovaries and includes any single or combination of endometritis, tubo-ovarian abscess, and salpingitis. PID has the highest incidence in ages 15-25, can lead to ectopic pregnancy and chronic pelvic pain, and is the leading

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cause of infertility in women. PID can be sexually transmitted (85% of cases) or naturally occurring (15%) with organisms from vaginal microbiota, enteric organisms (e.g., *Escherichia coli, Bacteroides fragilis*, Group B streptococci, and *Campylobacter* spp) or respiratory pathogens that have colonized the lower genital tract. Clinical diagnosis remains the most important practical approach but can be clinically difficult to identify when patients present with mild or nonspecific symptoms. Difficulty in diagnosis, low NPV of tests, and significant potential sequelae should make the threshold for therapy low. Finding symptoms on physical exam (cervical motion tenderness) as well as other criteria (elevated temperature or mucopurulent discharge) increases the positive predictive value of laboratory tests. Testing for CT/NG and consideration for syphilis and HIV are recommended in patients with PID as is a pregnancy test to rule out ectopic pregnancy in those with pelvic pain. Bacterial culture tests performed on specimens collected in a non-sterile manner (endocervical or dilatation and curettage [D and C]) have limited utility in diagnosing PID [390].

Actinomyces spp is part of normal oropharynx, gastrointestinal and urogenital tract microbiota and can often be seen on Pap smears. Approximately 7% of women using an IUD may have a finding of Actinomyces-like organisms on a Pap smear. In the absence of symptoms, women do not need IUD removed or antimicrobial treatment. Infection occurs most commonly in 2 settings; patient has an infection at the time of insertion of IUD and if the IUD is left in place past the recommended time of removal [391]. If Actinomyces infection is suspected, the laboratory should be notified to culture such samples anaerobically, including an anaerobic broth that is held for ≥ 5 and up to 14 days.

Postpartum endometritis should be suspected when the patient presents with high fever (≥ 101°F or > 100.4°F (38.0°C) on more than two occasions > 6 h apart after the first 24 h of delivery and up to 10d post-delivery), abdominal pain, uterine tenderness and foul lochia. Usually a polymicrobic syndrome, the infection is most commonly seen in patients with unplanned caesarean section because of the inability to introduce antibiotics quickly. Reduction in postpartum endometritis has been shown when treating symptomatic BV in the late stages of pregnancy, as BV has been associated with preterm labor and prolonged delivery [392]. Late postpartum endometritis suggests possible CT or other chronic STI. Although the role of culture in the setting of endometritis is controversial, diagnostic tests, such as blood cultures, aerobic/anaerobic cultures and histology can be considered in the diagnosis of PID and postpartum endometritis (Table 44).

Special Populations with Suspected STI

Children for whom sexual abuse or assault is a consideration should be referred to a setting or clinic that specifically deals with this situation. Molecular forensic data for STI diagnostics in children under 14 (CT, NG, TV) is needed to support optimal testing methods. Readers are referred to the Pediatric Redbook, published case studies and CDC guidelines where NAATs are recommended because of performance parameters as well as ability to use non-invasive specimens [204, 379].

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In pregnant patients, screening for HIV, syphilis, hepatitis B surface antigen (HBsAg), CT, NG, and HCV if prevalence is >0.1%., should be performed regardless of previous testing. Some states require 3rd trimester screening for HIV and routine syphilis serologic screening of pregnant women at first prenatal visit, at 28 weeks' gestation and at delivery for women who live in communities with high rates of syphilis, women with HIV infection, or those who are at increased risk for syphilis acquisition [204].

AAP and ACOG recommend universal antepartum screening for GBS to help decrease early onset of neonatal sepsis and to decrease empiric treatment based on risk, which leads to unnecessary antibiotics in GBS-negative females [393]. The screening interval has shifted in gestation from 35-37 weeks to 36 0/7 - 37 6/7. A single flocked swab is obtained from the vagina first and then from the rectum and placed in a liquid-based transport medium such as Amies. Swabs incubated in selective enrichment broth are recommended for culture and/or NAATs. Direct antepartum testing with NAATs is not recommended by ASM [394]. The finding of mutant GBS strains harboring different chromosomal deletions in or near the region that encodes the *cfb* gene may escape detection by molecular assays targeting the *cfb* gene alone and the method for detecting GBS by the Christie-Atkins-Munch-Petersen (CAMP) test also show decreased sensitivity [395]. Susceptibility testing of GBS is not routinely performed and recommended only in patients allergic to penicillin

Past history of STIs, pregnant patients in higher risk groups, and/or clinical presentation consistent with infection, should be assessed for other pathogens as warranted, e.g., HSV if vesicular lesions are present [396]. For women diagnosed with HIV during pregnancy, refer to specific testing guidelines. Virologic assays (i.e., HIV RNA or HIV DNA NAATs) that directly detect HIV must be used to diagnose HIV in infants and children aged <18 months with perinatal and postnatal HIV exposure. HIV antibody and HIV antigen/antibody tests should not be used [397].

Table 41. Laboratory Diagnosis of Genital Lesions

Etiologic Agents	Diagnostic Procedures	Optimum Specimen	Transport Issues
Herpes simplex virus 1 and 2 Note: in children with genital lesions, consider atypical VZV	NAAT[1]	Lesion swab (cutaneous, mucocutaneous) in UTM, VTM[2]	Transport conditions vary depending on test methodology, check with laboratory criteria[3]
	Serology[4]	Serum	Clot tube, RT 2-24h

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Orthomyxoviridae (mpox and variola) Monkeypox virus	NAAT[5]	Lesion swab in UTM/VTM Combine if same source, separate tubes if different source (e,g, skin, rectum)	RT 24 h, 7 days refrigerated
Condyloma acuminata (**genital warts**) i[6]	Histopathology; hrHPV testing not done on warts	Biopsy or shaving	Formalin container, RT, 2-24 h
Syphilis *(Treponema pallidum)*	Non–Treponemal Serology (VDRL or RPR)[7]	Serum	Clot tube, RT, 2 h-24h
	Treponemal Serology EIA/ CIA or TP-PA, FTA-ABS)[8,9]	Serum	Clot tube, RT, 2 h-24h
Chancroid (*Haemophilus ducreyi*) [10]	Culture send out[11]	Swab of lesion base without surface genital skin	Call lab if culture is required so lab can consult with reference lab and inform on specimen collection and transport needed
Lymphogranuloma venereum (LGV)[10] (*Chlamydia* serovars L1, L2, L2a, L2b, L3)	Serology Microimmunofluorescence (MIF)[12]	Serum	RT, 2 h
	NAAT[13]	Rectal swab if proctitis, check with lab if other sources available	RT 2 days; or refrigerate
Granuloma inguinale[10] (donovanosis) *Klebsiella granulomatis*	Giemsa or Wright stain in conjunction with anatomic pathology. Visualization of blue rods with prominent polar granules	Scraping of lesion base into formalin (send to Anatomic pathology section of laboratory)	RT, 2 h -48h
Scabies	Microscopic visualization, magnifying lens or	Collect parasite from skin scrapings into sterile petri	RT, 48 h

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	lighted videoscope at bedside or in the clinic	dish/slide to send to microbiology laboratory[14]	
Lice	Macroscopic identification lice/nits	Collect parasite/hair into sterile petri dish/cup	RT, 48h
Human Papilloma Virus (HPV) Refer to text[15] Specific guidelines and triaging recommendations vary based on age and test(s) performed by laboratory	1.Cytology alone (≥21-30) 2.Co-testing cytology plus HPV (≥25-30) 3. HPV screening alone (≥30) 4.Primary HPV screening ≥ 25	Endocervical brush into liquid cytology medium or vaginal swab in transport tube[16]	RT, 24-48 h

[1]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 and DFA which are no longer commonly performed, especially when lesions are past vesicular stage.

[2]VTM – viral transport medium or UTM – universal transport medium. Check with laboratory, most are maintained and shipped at RT, ice not required.

[3]Collection and transport systems used by laboratories generally allow storage at RT 24-48 h, 7 days refrigerated. Freezing may not be on label for specific tests and freeze thaw may compromise results. Labs are encouraged to clarify specimen collection and transport parameters for specific assays being use and communicate to clinicians. As well, criteria that may result in the need to recollect.

[4] Serology appropriate in limited cases. Serology can be non-specific for HSV-1 and HSV-2 differentiation; should be limited to patients with clinical presentation consistent with HSV but negative by NAAT; request type-specific immunoglobulin G (IgG)-based assays that differentiate HSV-1 and HSV-2; , as HSV-2 has a higher likelihood of recurrence

[5]As of 1/20/2023, mpox Emergency Use Authorization (EUA) exists for NAATs available through the LRN, commercial laboratories and laboratories performing LDTs.

[6]The diagnosis of genital warts is most commonly made by visual inspection

[7]Non-treponemal tests – (rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL); less sensitive in early and late disease; typically become negative after treatment; do not use to test pregnant patients due to potential for false-positive results. Use non-treponemal tests to follow titers after treatment. Positive patients may have serologic responses that do not have a four-fold change in titer (for example,1:16 to 1:4) or fail to serorevert and persist despite treatment, considered an inadequate serologic response. Serial testing should be done by same test and through same laboratory to get optimal results. Presumptive treatment is recommended in patients with risk factors for syphilis.

[8]Treponemal tests – Enzyme immunoassay (EIA) or chemiluminescence immunoassays (CIA) 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.

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

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[10]Uncommon genital ulcers in the US are typically diagnosed by clinical presentation, risk factors, and exclusion of syphilis, HSV and mpox. HIV testing should be part of workup if not known.
[11]Gram stain with chancroid organisms shows small rods or chains in parallel rows, "school of fish"; culture requires special media and culture sensitivity only 30-70%. Testing should only be performed by laboratory that regularly performs this testing, check with routine laboratory so that they can clarify with reference laboratory correct collection and transport criteria if culture is needed.
[12]MIF titers ≥ 256 with appropriate clinical presentation suggests LGV
[13]NAATs for CT will detect L1-L3 but do not distinguish these from the other CT serovars, typical lesion sites not FDA-cleared, some labs have validated rectal swabs, NAAT performed through CDC in outbreak situations [355].
[14]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 or sterile petri dish (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 several papules.
[15]High-risk HPV (hrHPV) testing currently recommended in women ≥ 25-30 years of age (see text). HPV testing is not routinely recommended for the diagnosis of HPV in a sexual partner or in patients ≤21y/o.
[16] HPV testing may require specific manufacturer specimen collection device and transport guidelines.

Table 42. Laboratory Diagnosis of Bacterial Vaginosis, Yeast Vaginitis, Trichomoniasis and Aerobic vaginitis

Common Etiologic Agents	Diagnostic Procedures [1]	Optimum Specimens	Transport Issues
Yeast (pH <4.5[2])	Saline wet mount[3] and 10% KOH[,4]	Swab of vaginal discharge	Submitted in 0.5 mL saline or transport swab, RT, 2 h
	Culture[5]	Swab of vaginal discharge	Submitted in transport swab, RT, 24 h
Bacterial vaginosis (BV) (pH >4.5[2])	Wet mount and 10% KOH, Whiff test performed at POC[6]	Swab of vaginal discharge	Submitted in 0.5 mL saline or transport swab, RT, 2 h
	Quantitative Gram stain[7] (Nugent scoring)	Swab of vaginal discharge	Place directly into transport swab, RT, 24 h
Trichomoniasis (pH >4.5[2])	Saline wet mount[8]	Swab of vaginal discharge	Submitted in saline, RT, 30 min (optimal) – 2 h
	Rapid antigen test[9]	Swab of vaginal epithelium/discharge	Submitted in transport swab

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			or saline, RT, 24 h
	NAAT[10]	Vaginal, endocervical swab, urine or liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	submitted in transport swab. RT 24h, 7 days refrigerated
Multiplex Vaginal Panels [11,12] 3 FDA-cleared and several commercial Lab-developed tests (LDT)	NAAT	Symptomatic, Self-collected or clinician collected Specific manufacturer transport containers Refer to laboratory test catalogue	RT 24 h, 7 days refrigerated
Vaginal Microbiome assessment LDT- may help for patients with chronic presentations of vaginitis	Metagenomic sequencing	Home-collected vaginal swab	Check with reference laboratory for transport conditions
Aerobic vaginitis[13]	Gram stain, wet mount, culture, metagenomic sequencing	Symptomatic, females, vaginal swab in transport swab tube	RT 24 h, refrigerated 7 days

[1] Diagnostic procedures that are rapid and performed either at the point of care or in the laboratory are included because not every laboratory or clinician site has access to NAATs. However, both clinicians and laboratorians should recognize the poor sensitivity and specificity of these POC tests and the absence of routine performance of all tests required to meet Amsel's criteria significantly compromises correct diagnosis and treatment of patients. For best possible quality of these results, training and competency should be assessed regularly for POC tests and/or consider discontinuing their performance.

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

[3]Sensitivity of wet mount between 40-80% (349)

[4]KOH – potassium hydroxide

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

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

[7]Quantitative Gram stain specific for BV, but 20-30% indeterminate [369, 371]. Do not perform culture or DNA probe test that only detects *G. vaginalis*, which yields increased false positive BV determinations and overtreatment [363]. For pregnant patients with symptomatic vaginitis, testing and treatment are recommended to reduce postpartum endometritis [392].

[8]Wet mount for trichomonads requires live organisms to visualize movement and has poor sensitivity (40-70%), specificity is not 100% as white blood cells may be misinterpreted as TV.

[9]OSOM® Trichomonas Rapid Test (Sekisui Diagnostics, Burlington, MA); does not require live organisms for optimal test performance, sensitivity ranges from 85– to 95% compared to culture and NAAT in symptomatic patients [345, 380].

[10]NAAT- nucleic acid amplification test. Many TV NAATs are currently FDA-cleared. Specific use (screening as well as diagnostic, female and/or male, specific sources and self-collection vary depending on manufacturer). Same specimen and collection device often used for CT/GC NAAT.

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[11]All multiplex vaginal panels include multiple targets for vaginal microbiota to diagnose BV and Candida spp, including strains more commonly associated with fluconazole resistant for VVC (e.g. C. glabrata). TV is not included on all panels and needs to be ordered as a separate test, but same sample can be used.
[12] Data from multiple studies have shown consistently, that multiplex vaginal panels more accurately diagnose BV, VVC and TV better than current SOC on site smear interpretation, in lab Gram stain Nugent Gram smear interpretation, and DNA hybridization test (Affirm VP III) [361, 365-368, 372-376]. For yeast see footnote 5.
[13] Entity different from BV, abnormal vaginal microbiota consisting of aerobic enterics or pathogens, GBS, *Enterococcus faecalis, S. aureus*. Vaginal inflammation, erosions and specific host response [398].

Table 43. Laboratory Diagnosis of Pathogens Associated with Cervicitis/Urethritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Chlamydia trachomatis (CT)	NAAT[1]	Urine, Endocervical, vaginal and/or urethral swab (rectum, pharynx, conjunctiva[2], liquid-based cytology[3])	Laboratory-provided transport device, RT, 2 d, refrigerated 7 days
Neisseria gonorrhoeae	Gram Stain [4]	Urethral discharge (male only)	Smear on slide directly or submit swab in transport medium, RT, immediately
	NAAT[1]	Urine, Endocervical, vaginal and/or urethral swab Rectal, pharynx, conjunctiva[2], liquid-based cytology specimen[3]	Laboratory-provided transport device, RT, 48 h, 7 days refrigerated
	Culture[5]	Endocervical, urethral, conjunctival, nasopharyngeal, pharynx, rectal swab	Transport medium, RT, ≤ 1 h Do not refrigerate specimen
Trichomonas vaginalis	Saline wet mount[8]	Endocervical or urethral swab	Submit in 0.5 mL saline, 30 min–2 h
	Rapid antigen test	Vaginal swab	Laboratory-provided transport device, RT, 24 h
	NAAT[1]	Vaginal, endocervical swab, urine and liquid-based cytology specimen, urethral, rectal, pharyngeal swabs	Laboratory-provided transport device, RT, 2 d

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Herpes simplex virus	NAAT[8]	Swab of lesion cutaneous, mucocutaneous, label site	Laboratory-provided transport device, consult laboratory
Mycoplasma genitalium	NAAT[9]	Urine or urethral	Laboratory-provided transport device, Assay specific

[1]NAAT – nucleic acid amplification tests are the SOC for CT and NG. Several FDA-cleared NAATs for CT, NG and TV exist. As well, some tests include other pathogen options, e.g. *M. genitalium*, and HSV 1 and 2. Look at specific manufacturer's intended use to identify if screening and/or diagnostic. Multiple specimen types, including extragenital sites, and a single transport can often be used for multiple STIs (NG, CT, TV). Provider needs to check with laboratory for availability.

[2] Conjunctival specimens typically collected on neonates for CT and/or NG are not FDA-cleared specimens. Check with lab if they have validated this source. If NAATs not available, culture by appropriate methods. Gram stain cannot be used for definitive diagnosis with conjunctival specimens as normal oral and/or vaginal microbiota may be present that is misleading.

[3]liquid-based cytology medium is not a commonly used specimen for NAATs. Check with laboratory if this is acceptable.

[4] Gram stain for NG in males only; 10-15 WBC/ HPF and intracellular gram-negative diplococci (gndc) 95% specific if no WBCs specificity is only about 30% [204]. Not as sensitive as NAATs

[5]Culture allows for antimicrobial susceptibility testing; vancomycin in media may inhibit some GC strains

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

[7]OSOM® Trichomonas Rapid Test (Sekisui Diagnostics, Burlington, 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. Not recommended for men.

[8]NAAT – nucleic acid amplification tests, currently many FDA-cleared. Check with lab on available sources validated and potential sex and age restrictions.

[9]NAATs for *Mycoplasma genitalium* (MG), are FDA-cleared for Aptima® (Hologic) and Alinity™ m system (Abbott), LDT performed in selected labs, check availability. Resistance detection for macrolides and ciprofloxacin in development [386]. Testing is recommended in men who have non-gonococcal urethritis (NGU) or recurrent NGU [204].

Table 44. Laboratory Diagnosis for Pathogens Associated with Pelvic Inflammatory Disease and Endometritis

Common Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Mixed anaerobic organisms Vaginal microbiota	Blood cultures to assess unusual causes of PID or endometritis	Blood, 2 separate sets, 20 mL each, venipuncture collections	Inject into blood culture bottles, RT

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Enterobacterales, enterococci Group A and B streptococci *Mycoplasma* *Actinomyces spp*[1]	Gram stain[2] Aerobic and anaerobic culture[3]	Aspiration of endometrium, tubo-ovarian abscess and/or fallopian tube contents	Place in or inject into sterile anaerobic container[4], RT, 30 min -1h
	Tissue for histological evaluation	Endometrial biopsy	Sterile container, RT, 30 min; Formalin container, RT, 30 min -24 h
Neisseria gonorrhoeae (GC)[5] *Chlamydia trachomatis* (CT) *Trichomonas vaginalis* *Mycoplasma genitalium*	NAAT	Urine, endocervical swab	Laboratory-provided transport device, RT-48 h
Human immunodeficiency virus (HIV)	HIV EIA-antibody	Serum	Clot tube, RT, 24 h

[1]*Actinomyces* spp is an uncommon cause of PID.

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

[3]Limited identification and antimicrobial susceptibility testing (AST) when cultures show multiple mixed aerobic and anaerobic organisms, or from non-sterile site

[4] Invasive specimens obtained by laparoscopic or other sterile technique

[5]Patients with late appearing post-partum endometritis consider chronic and/or asymptomatic STI such as CT, NG.

XIII. BONE AND JOINT INFECTIONS

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

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

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Key points for the laboratory diagnosis of bone and joint infections

- Swabs are not recommended for specimen collection, with synovial fluid and/or tissue biopsies being recommended
- Blood cultures are indicated for detection of some agents of osteomyelitis and native joint infection, but usually not for periprosthetic joint infection diagnosis
- Joint fluids should ideally be cultured in aerobic and (specimen volume permitting) anaerobic blood culture bottles
- For periprosthetic joint infection diagnosis, 3-4 separate tissue samples should be submitted for aerobic and anaerobic culture; sonication of explanted prostheses followed by semi-quantitative aerobic and anaerobic culture of the resultant sonicate fluid may be used to detect pathogens
- Fungal and mycobacterial stains and cultures should not be routinely performed for the diagnosis of periprosthetic joint infection
- When anaerobic bacteria are suspected, anaerobic transport containers should be used for transportation of tissues and fluids to the laboratory
- Some agents of bone and joint infection are non-culturable or poorly culturable and require molecular and/or serologic methods for detection

Osteomyelitis

Osteomyelitis can occur following hematogenous spread, after a contaminated open fracture, or following direct inoculation of microorganisms into bone with surgery or trauma; those with underlying diabetes mellitus or vascular insufficiency are at particular risk for osteomyelitis. Vertebral osteomyelitis/spondylodiskitis is addressed below. Osteomyelitis is typically suspected on clinical grounds, with confirmation involving imaging, and microbiologic and histopathologic tests. The peripheral white blood cell count may be elevated; erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are often elevated as well. Establishing an etiologic diagnosis, which is important for directing appropriate clinical management since this varies by microorganism-type and associated antimicrobial susceptibility, nearly always requires obtaining bone for microbiologic evaluation (unless blood cultures are positive in the context of convincing radiographic findings). This can be accomplished by imaging-guided or surgical sampling. As much specimen as possible should be submitted to the laboratory; specimens may include pieces of intact bone, shavings, scrapings and/or excised or aspirated necrotic material (Table 45). Swabs are not recommended. Cultures of sinus tracts are generally not recommended, because recovered organisms, aside from *Staphylococcus aureus*, do not correlate with those found in deep cultures.

Hematogenous osteomyelitis is usually monobacterial, whereas that resulting from contiguous infection is often polymicrobial. Acute hematogenous osteomyelitis of long bones mainly occurs in prepubertal children, but may occur in older individuals, persons who inject drugs, and those with indwelling central venous catheters. In prepubertal children, the most common

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microorganisms involved are *S. aureus* and *Streptococcus pneumoniae*; *Kingella kingae* is common in children under the age of 4 years [399]. Osteomyelitis in neonates, especially in those with indwelling central venous catheters, typically results from hematogenous spread; commonly involved organisms include *Streptococcus agalactiae* and aerobic Gram-negative bacteria, especially *Escherichia coli*. *Candida* species and *Pseudomonas aeruginosa* are more commonly encountered in persons who inject drugs and those with indwelling central venous catheters. In children, diagnosis is often (but not always) made based on clinical and imaging findings in the context of positive blood cultures (https://www.idsociety.org/practice-guideline/bone-and-joint-infections---osteomyelitis/). In adults, imaging-guided aspiration or open biopsy is typically necessary.

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

In patients with diabetes, osteomyelitis typically involves the foot as a complication of a chronic foot ulcer; a positive probe-to-bone test is associated with osteomyelitis. Specimens for bone culture (aerobic and anaerobic) and histology can be obtained by open debridement, needle puncture, or transcutaneous biopsy. Readers are referred to a guideline that provides greater detail on the diagnosis of diabetic foot infections [400].

Vertebral osteomyelitis/disk space infection/spondylodiskitis is often hematogenous in origin (e.g., from skin and soft tissue, urinary tract, intravascular catheter, pulmonary infection sites), but can occur postoperatively or following a procedure. *S. aureus* and coagulase-negative staphylococci are most commonly involved, followed by Gram-negative bacilli, streptococci, *Candida* species, and in patients with relevant risk factors, *M. tuberculosis* (and occasionally non-tuberculous mycobacteria) and *Brucella* species. Two sets of aerobic and anaerobic blood cultures and ESR and CRP should be obtained; in addition, *Brucella* blood cultures and serologic tests should be obtained in those in areas endemic for brucellosis, fungal blood cultures in those with relevant epidemiologic or host risk factors, and a purified protein derivative test and interferon-γ release assay considered in those at risk for tuberculosis. Patients suspected of having native vertebral osteomyelitis based on clinical, laboratory and imaging studies, with *S. aureus*, *Staphylococcus lugdunensis* or *Brucella* bloodstream infection or, in an endemic setting, a positive *Brucella* serology, do not need further testing. For all others, imaging-guided aspiration/biopsy of a disc space or vertebral endplate is recommended, with specimens submitted for Gram stain and aerobic and anaerobic culture and, if adequate tissue can be obtained, histopathology. If results are negative or inconclusive (e.g., *Corynebacterium* species is isolated), a second imaging-guided aspiration biopsy, percutaneous endoscopic discectomy and drainage procedure, or open excisional biopsy, should be considered to collect additional specimens for repeat and additional testing. Readers are referred to a guideline that provides greater detail on the diagnosis of native vertebral osteomyelitis in adults [401]. Further studies are needed to define ideal diagnostic approaches for vertebral osteomyelitis.

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Molecular diagnostics may be performed on bone biopsies but are not considered first-line diagnostic tests. Microorganism-specific NAATs or, a broader approach such as 16S ribosomal RNA gene PCR/sequencing (for bacterial detection) [402], may be considered. One strategy is to temporarily set aside a specimen with this type of testing performed if cultures are negative and other findings point to a diagnosis of infection; another is to perform molecular testing on formalin-fixed paraffin embedded tissue collected for histopathologic evaluation. *K. kingae* may require molecular detection methods for diagnosis, with *K. kingae* PCR or 16S ribosomal RNA gene PCR/sequencing [402].

Infections of Native Joints

Joints can be hematogenously seeded by bacteria, or seeded by direct inoculation or from a contiguous focus, with a majority of infections being monoarticular. *S. aureus*, *Streptococcus* species and *Neisseria gonorrhoeae* are common causes of septic arthritis of native joints, followed by Gram-negative bacilli, which mainly cause septic arthritis in neonates, older individuals, persons who inject drugs, and the immunocompromised, and other Gram-positive bacteria. *K. kingae* is the most common etiology of bacterial joint infection in children younger than 4 years. Viruses, including parvovirus B19, chikungunya virus, and rubella, among others, may be associated with arthritis (Table 46). Subacute or chronic infectious arthritis may be caused by *M. tuberculosis* and nontuberculosis mycobacteria, *Borrelia burgdorferi, Candida* species*, Blastomyces dermatitidis, Coccidioides immitis/posadasii, Histoplasma capsulatum, Sporothrix schenckii, Cryptococcus neoformans/gattii,* and *Aspergillus* species*,* among others. Septic bursitis, which usually involves the prepatellar, olecranon or trochanteric bursae, is usually caused by *S. aureus.*

Although peripheral blood white cell count, ESR, and CRP are often elevated, they are nonspecific. Arthrocentesis of a septic joint usually reveals purulent, low-viscosity synovial fluid with an elevated neutrophil count. Traditionally, a synovial fluid leukocyte count more than 50,000 cells/mm^3 was considered to suggest septic arthritis; however, lower counts do not exclude the diagnosis. Ideally, synovial fluid from native joints should be submitted for Gram stain, and cultured in aerobic and anaerobic blood culture bottles. If synovial fluid Gram stain and culture are negative, molecular testing of synovial fluid with a multiplex PCR panel or 16S ribosomal RNA gene PCR/sequencing [402], should be considered; biopsy of the synovium for Gram stain, aerobic and anaerobic cultures, and histopathologic evaluation, with or without fungal and mycobacterial stains and cultures may be considered. Concomitant or secondary bacteremia or fungemia occurs sporadically in patients with septic arthritis; thus, blood cultures collected during febrile episodes are recommended. Some less common agents, such as *K. kingae*, may require molecular detection methods for optimal diagnosis; *K. kingae* PCR is available as part of a recently FDA-cleared multiplex PCR panel for testing synovial fluid and offered by individual laboratories as single- or multiple- target laboratory developed tests. *N. gonorrhoeae* is also included as part of the recently FDA-cleared multiplex PCR panel for testing synovial fluid.

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Periprosthetic joint infection

A special category of infection exists for periprosthetic joint infection, which may involve knee, hip, shoulder, elbow or other prostheses [403]. Staphylococci, including not just *S. aureus,* but also the coagulase-negative staphylococci, especially *Staphylococcus epidermidis*, are particularly common causes, but many other organisms, including streptococci, enterococci, aerobic Gram-negative bacilli, anaerobic bacteria (e.g., *Cutibacterium acnes, Finegoldia magna*) and fungi, can be involved (Table 47). *C. acnes* is particularly common in shoulder arthroplasty infection.

The diagnosis of periprosthetic joint infection and definition of its microbiology is ideally made pre-operatively, but if this is not possible, diagnosis and, if present, definition of microbiology should be pursued at the time of revision or resection arthroplasty. Readers are referred to recently published guidance on the diagnosis of periprosthetic joint infection [404-406]. Preoperatively, ESR and CRP are recommended (some also use D-dimer), as is arthrocentesis for synovial fluid cell count and differential and culture, ideally in aerobic and anaerobic blood culture bottles. Criteria for the interpretation of synovial fluid cell count and differential in the presence of a prosthetic joint differ from those in the absence of a prosthetic joint. Synovial fluid alpha-defensin [407], and intraoperative frozen section analysis are reliable diagnostic tests for periprosthetic joint infection. For tissue culture, multiple specimens should be submitted for aerobic and anaerobic cultures, four if using conventional plate and broth cultures and three if culturing tissues in aerobic and anaerobic blood culture bottles [408]. Tissue can be processed in several ways, including crushing, stomaching, and bead mill processing using glass beads [409]. Two or more intraoperative cultures or a combination of preoperative aspiration and intraoperative cultures that yield the same organism is considered definitive evidence of periprosthetic joint infection. Notably, single positive tissue or synovial fluid cultures, especially for organisms which may be contaminants (e.g., coagulase-negative staphylococci, *C. acnes*), should not be considered evidence of definite infection. Gram stain is not recommended. Isolation of *C. acnes* may require culture incubation times as long as 14 days. The pathogenesis of periprosthetic joint infection relates to the presence of microorganisms in biofilms on the implant surface. Therefore, if the arthroplasty is resected, the implant components may be vortexed and sonicated and the resultant sonication fluid semi-quantitatively cultured [410]. Since fungi and mycobacteria are extremely rare in this setting, they should not be routinely sought.

Table 45. Microbiology Laboratory Diagnosis of Osteomyelitis[1]

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Staphylococcus aureus Coagulase-negative staphylococci *Salmonella* species[2] *Streptococcus* species[3] *Enterococcus* species Enterobacterales *Candida* species	Gram stain Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing[9]	Bone biopsy	Sterile anaerobic transport container, RT, 2 h

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Brucella species[4] *Pseudomonas* species[5] Anaerobic bacteria			
Kingella kingae	Gram stain Aerobic bacterial culture *K. kingae* NAAT 16S ribosomal RNA gene PCR/ sequencing[9]	Bone biopsy	Sterile container, RT, immediatelyi
Mycobacterium tuberculosis[6]	Acid fast smear Mycobacterial culture *M. tuberculosis* NAAT[6] 16S ribosomal RNA gene PCR/ sequencing[9]	Bone biopsy	Sterile container, RT, 2 h
Blastomyces dermatitidis *Coccidioides immitis/posadasii*	Calcofluor-KOH stain Fungal culture Serology Urine antigen	Bone biopsy	Sterile container, RT, 2 h
Mixed aerobic and anaerobic bacterial microbiota of the oral cavity including *Actinomyces* species in patients with maxillary or mandibular osteomyelitis[7]	Gram stain Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing[9]	Bone biopsy	Sterile anaerobic transport container, RT, 2 h
Mixed bacterial microbiota in diabetic patients with skin and soft tissue extremity infections	Gram stain Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing[9]	Bone biopsy	Sterile anaerobic transport container, RT, 2 h
Nocardia species, other aerobic actinomycetes and soil filamentous fungi in patients with mycetoma[8]	Gram stain Aerobic bacterial culture *Nocardia* stain Calcofluor-KOH stain *Nocardia* culture Fungal culture	Bone biopsy	Sterile container, RT, 2 h

[1]Histopathologic assessment should be performed.

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

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

[4]*Brucella* species may be recovered in standard aerobic bacterial cultures (prolonged incubation may be required); the laboratory should be notified when *Brucella* species is considered a potential cause of osteomyelitis so that cultures are examined only in a biological safety cabinet. Concomitant blood cultures and serology testing are recommended. *Brucella* species may be detected by 16S ribosomal RNA gene PCR/sequencing.

[5]Hematogenous osteomyelitis caused by *Pseudomonas aeruginosa* and other *Pseudomonas* species occurs most often in persons who inject drugs. *P. aeruginosa* is the most common bacterial cause of calcaneal osteomyelitis in individuals who develop this infection after stepping on nails while wearing sneakers.

[6]The most common site of osteomyelitis due to *M. tuberculosis* is vertebral bodies. *M. tuberculosis* also represents a common cause of clavicular osteomyelitis. Commercial NAATs are not FDA-cleared for non-respiratory sites, so a laboratory-developed/validated test must be used if NAATs are requested. *M. tuberculosis* may be detected by 16S ribosomal RNA gene PCR/sequencing.

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[7]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 microbiota of the oral cavity and may be either monomicrobial or polymicrobial. *Actinomyces* species are recognized pathogens in this setting; when *Actinomyces* species are suspected, specimens should be transported to the laboratory under anaerobic conditions and cultures incubated for 10-14 days.
[8]Mycetoma is a chronic soft tissue infection of the extremities which can extend into contiguous bone and connective tissue. It occurs most often in tropical and subtropical climates and may be characterized by the development of draining sinuses. Etiologic agents are derived from the soil. Sinus tract drainage material, when present, may be representative of the etiology of underlying osteomyelitis. In addition to the stains and cultures noted in the table, sinus drainage may 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* species as a pathogen so that appropriate media (e.g., Middlebrook agar, Sabouraud's dextrose agar) can be inoculated which facilitate recovery of this organism.
[9]May be performed on formalin-fixed paraffin embedded tissue. 16S ribosomal RNA/sequencing or a multiplex PCR panel may be considered if cultures are negative.

Table 46. Microbiology Laboratory Diagnosis of Joint Infection[1]

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Acute Arthritis			
Staphylococcus aureus *Staphylococcus lugdunensis* *Streptococcus* species Enterobacterales *Pseudomonas* species *Kingella kingae*[2] *Neisseria gonorrhoeae*[3]	Gram stain Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing[4] Multiplex NAAT[4] Blood culture	Synovial fluid (synovium biopsy) Blood	Sterile container, RT, 2h (Inoculate fluid into aerobic and anaerobic blood culture bottles) Aerobic and anaerobic blood culture bottles
Brucella species	*Brucella* serology Culture[5] Blood culture[5] 16S ribosomal RNA gene PCR/ sequencing[4]	5 mL serum Synovial fluid and/or synovium biopsy Blood	Clot tube, RT, 2 h Sterile container, RT, 2h (Inoculate fluid into aerobic blood culture bottle) Aerobic blood culture bottle Sterile container, RT, 2h

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Parvovirus-B19	Parvovirus-B19 serology Parvovirus-B19 NAAT	5 mL serum Synovial fluid	Clot tube, RT, 2 h Closed container, RT, 2 h
Rubella	Rubella serology	5 mL serum	Clot tube, RT, 2 h
Subacute or Chronic Arthritis			
Chikungunya	Chikungunya serology	5 mL serum	Clot tube, RT, 2 h
Borrelia burgdorferi	Lyme serology *B. burgdorferi* culture[6] *B. burgdorferi* NAAT 16S ribosomal RNA gene PCR/ sequencing[4]	5 mL serum Synovial fluid	Clot tube, RT, 2 h Sterile container, RT, 2 h
Mycobacterium tuberculosis Non-tuberculous mycobacteria	Acid fast smear AFB culture *M. tuberculosis* NAAT[7] 16S ribosomal RNA gene PCR/ sequencing[4]	Synovial fluid and/or synovium biopsy	Sterile container, RT, 2 h
Candida species *Cryptococcus neoformans/gattii* *Blastomyces dermatitidis* *Coccidioides immitis/posadasii* *Aspergillus* species	Calcofluor-KOH stain Fungal culture Serum cryptococcal antigen *Blastomyces dermatitidis* serology *Coccidioides immitis/ posadasii* serology Beta-d-glucan, galactomannan	Synovial fluid and/or synovium biopsy 5 mL serum	Sterile container, RT, 2 h Clot tube, RT, 2 h
Septic Bursitis			
Staphylococcus aureus	Gram stain Aerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing[4]	Bursa fluid	Sterile container, RT, 2 h

[1]Synovial fluid cell count and differential should be performed.

[2]*K. kingae* is the most common cause of septic joint infections before the age of 4 years. It does not grow well in culture. It is included in an FDA cleared multiplex NAAT panel for testing synovial fluid. It can alternatively be detected by a standalone *K. kingae* NAAT or 16S ribosomal RNA gene PCR/sequencing.

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[3]*N. gonorrhoeae* culture of synovial fluid may be negative. *N. gonorrhoeae* is included in a recently FDA cleared multiplex NAAT panel for testing synovial fluid. It can alternatively be detected by 16S ribosomal RNA gene PCR/sequencing. NAATs for *N. gonorrhoeae* may be performed on genitourinary sites and/or freshly voided urine and, if clinically indicated, rectal and oropharyngeal swabs, and culture for *N. gonorrhoeae* may be performed on specimens from genitourinary sites and, if clinically indicated, rectal and oropharyngeal swabs.
[4]16S ribosomal RNA gene PCR/sequencing or a multiplex PCR panel may be considered if cultures are negative.
[5]*Brucella* species may be recovered in standard aerobic bacterial cultures (prolonged incubation may be required); the laboratory should be notified when *Brucella* species is considered a potential cause of joint infection so that cultures are examined only in a biological safety cabinet. Concomitant blood cultures and serology testing are recommended. *Brucella* species may be detected by 16S ribosomal RNA gene PCR/sequencing.[6]Serology would be expected to be positive in the case of a positive culture or NAAT. Culture for *B. burgdorferi* requires specialized media, rarely results in recovery of the organism and is seldom done except in research settings. *B. burgdorferi* may be detected by 16S ribosomal RNA gene PCR/sequencing.
[7]Detection of *M. tuberculosis* or other *Mycobacterium* species by microscopy or culture is uncommon from synovial fluid in patients with joint infections due to these organisms. Analysis of synovial tissue enhances the likelihood of detection *M. tuberculosis* may be detected by 16S ribosomal RNA gene PCR/sequencing.

Table 47. Microbiology Laboratory Diagnosis of Periprosthetic Joint Infection.

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Staphylococcus aureus Coagulase negative staphylococci *Enterococcus* species *Streptococcus* species Enterobacterales *Pseudomonas aeruginosa* *Corynebacterium* species *Cutibacterium acnes* *Finegoldia magna* Other aerobic or anaerobic bacteria (Fungi) (Mycobacteria)	Aerobic and anaerobic bacterial culture[1] 16S ribosomal RNA gene PCR/ sequencing[5] Multiplex NAAT5 Aerobic and anaerobic bacterial culture[1] 16S ribosomal RNA gene PCR/ sequencing[3] Aerobic and anaerobic bacterial culture 16S ribosomal RNA gene PCR/ sequencing5 Gram stain not useful (any specimen type) Fungal and mycobacterial stains and cultures not first-line	Synovial fluid[2] Tissue biopsy samples (3-4) Prosthesis (if removed) submitted for vortexing/sonication with aerobic and anaerobic culture of sonicate fluid	Sterile anaerobic transport container, RT, 2 h

[1]Incubate anaerobic cultures up to 14 days for recovery of *C. acnes*, if clinically indicated (e.g., shoulder arthroplasty infection). Inoculate synovial fluid and tissues into blood culture bottles for culture for ideally recovery [411].
[2]Synovial fluid cell count and differential should also be performed. Alpha-defensin testing of synovial fluid may be considered.
[3]May be performed on formalin-fixed paraffin embedded tissue.
[4]Histopathologic assessment of acute inflammation should be performed.
[5]16S ribosomal RNA gene PCR/sequencing or a multiplex PCR panel may be considered if cultures are negative

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XIV. BLOOD AND TISSUE PARASITE INFECTIONS

Blood and tissue parasites comprise a large number of protozoa and helminths found in both tropical and temperate climates worldwide [412-416]. Some parasitic infections are associated with high morbidity and mortality (e.g., malaria, amebic encephalitis) while others cause only mild or asymptomatic disease (e.g., filariasis due to *Mansonella* spp, toxoplasmosis in immunocompetent adults). As expected, the most commonly submitted specimens for laboratory identification of these parasites are whole blood, tissue aspirates/biopsies, and serum for serologic studies.

Microscopy remains the cornerstone of laboratory testing for the identification of most blood parasites and many tissue parasites [414, 415]. 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 of the microfilariae of the nematodes *Brugia, Wuchereria*, *Loa loa* and *Mansonella*, whereas microscopic examination (microbiology, cytopathology, and histopathology preparations), *in vivo* and *in vitro* culture and/or nucleic acid amplification of ulcer samples, bone marrow, tissue aspirates, and biopsies may be useful in the diagnosis of other parasitic diseases such as African trypanosomiasis, onchocerciasis, trichinosis, toxoplasmosis, and leishmaniasis. Although requiring a minimal number of reagents and equipment, the accuracy of microscopic methods requires well-trained and experienced laboratorians. 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 (e.g., for trypanosomes), as well as centrifugation or filtration of liquid specimens (e.g., Knott's concentration for detecting microfilariae in blood). Additionally, artificial intelligence (AI) algorithms can be employed for enhancing parasite detection in blood and stool specimens [417]. In general, AI-assisted testing aims to improve the efficiency of the specimen examination process by laboratory technologists and has the potential to increase the sensitivity and reproducibility of parasite detection as compared to manual interpretation. These promising tools have already been implemented in some specialized clinical laboratories.

Regardless of the final means of processing and analysis, samples must be properly obtained, transported to the laboratory as quickly as possible and processed in a timely fashion to preserve organism viability and/or morphology. Organism viability and morphology may be adversely affected by a number of different factors including temperature, humidity and exposure to fixatives or anticoagulants. Transportation requirements are described for each organism in the corresponding sections below.

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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 [414, 415]. Additionally, the sensitivity of serologic testing may vary by the extent and anatomic location of disease in the host. 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 may provide varying results. In contrast, EIAs typically provide only qualitative positive or negative results determined by a 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. In some cases, it is desirable to confirm the result of an EIA by using a more specific immunoblot assay. Further information is provided for specific parasites in the sections below and in Table 48.

Laboratory methods that detect parasite antigens and/or nucleic acid provide an attractive alternative to traditional morphologic and serologic techniques for certain parasitic diseases. For example, a simple rapid immunochromatographic card assay for the detection of *Plasmodium* (BinaxNOW® Malaria, Alere™, Waltham, MA) has been cleared by the FDA for *in vitro* diagnostic use and many more assays are commercially available for this purpose outside of the United States [418]. At this time, the BinaxNOW Malaria remains the only FDA-cleared/approved assay for malaria antigen detection. These rapid detection tests (RDTs) are particularly useful in acute care settings such as emergency departments or out-patient clinics to establish a diagnosis of malaria quickly while awaiting results of confirmatory blood films. These tests are also commonly used during times when personnel with sufficient expertise to screen and interpret blood films for parasites is not available (e.g., the night shift). In general, most malaria RDTs, including the BinaxNOW Malaria, are adequately sensitive in typical patients with symptomatic malaria ("fever and chills") but lose sensitivity when the parasitemia is very low or infection is due to non-*falciparum* species [418]. Additional information is available in the section on malaria below.

Finally, the Centers for Disease Control and Prevention [415] and a number of reference laboratories in the United States, Canada and Europe perform extremely sensitive nucleic acid amplification tests (NAATs) such as real-time polymerase chain reaction (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

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time from specimen collection to receipt of result may be longer than desired for optimal patient care. In situations where infection is potentially life threatening, initial testing should be performed locally and empiric treatment should be considered while awaiting results from the outside laboratory.

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

- Microscopy is the cornerstone of laboratory identification but is highly subjective and dependent on technologist experience and training
- Proper specimen collection and transport are essential components of morphology and culture-based techniques.
- Serology shows significant cross-reactivity among helminths, including filariae
- Malaria antigen detection tests provide rapid and sensitive detection for symptomatic *P. falciparum* infection, but may fail detect low levels of infection, *P. falciparum* strains with HRP-2 deletions, and infection with non-*P. falciparum* species
- Automated hematology analyzers may fail to detect malaria or babesiosis parasites; request manual stain and evaluation if either agent is suspected
- NAATs are useful for detection of low parasitemia or in specifically identifying organisms which cannot be differentiated microscopically
- Antigen and nucleic acid detection methods should not generally be used to monitor response to therapy, since antigen or DNA may be detectable for days to weeks after successful treatment
- NAATs for detecting blood and tissue parasites are currently available only from specialized laboratories and turnaround time may be prolonged

Table 48 presents an inclusive overview of the approach to the diagnosis of blood and tissue parasitic infections based on published recommendations [414, 415]. Important points are bolded. Subsequent sections 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 and reference laboratories are more likely than community laboratories to have the experience and volume of specimens to properly validate the more esoteric tests for parasitic infections, as many are rare in the United States. These laboratories provide an important role in providing high impact testing that may not be available locally, and often deliver results within 1-2 days following receipt. When indicated, direct communication by phone or e-mail may hasten specimen processing and result reporting, especially when there is an urgent clinical situation. The DPDx website at CDC (https://www.cdc.gov/dpdx/diagnosticprocedures/index.html) provides a list of currently available diagnostic tests for parasitic infections available from the CDC. The CDC also provides a valuable

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telediagnostic consultation service that can be accessed through the DPDx website for both the laboratorian and clinician.

Babesia and Malaria

Babesiosis is caused primarily by *Babesia microti* in the U.S. and *B. divergens* in Europe [418]. A smaller number of infections occurring in California and Washington are due to *B. duncani*, while *Babesia divergens*-like organisms including the MO-1 strain have been detected in patients residing in Missouri, Kentucky, Washington, and Arkansas. Human malaria is caused by four species in the *Plasmodium* genus: *P. falciparum, P. vivax, P. ovale,* and *P. malariae* [418, 419]. The simian parasite *P. knowlesi* has also been reported to cause a significant portion of human cases in parts of Southeast Asia [419]. Table 49 summarizes the laboratory tests available for these agents.

The gold standard method for diagnosis of both malaria and babesiosis is microscopic examination of Giemsa-stained thick and thin blood films [419, 420]. Although this method requires a minimum number of resources (staining materials and high-quality, well-maintained microscopes), skilled and experienced technologists must be available to obtain maximum accuracy and efficiency [420]. 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. Samples should be obtained from fresh capillary or EDTA venous blood and slides prepared and read **as soon as possible** [420]. It is optimal for both thick and thin blood films to be prepared and examined.

The thick blood films are the most sensitive method for microscopic screening and allows detection of very low levels of parasitemia (less than 0.001% of RBCs infected) [419]. Use of the "scratch method" allows for improved adherence of the thick film to the slide and facilitates rapid examination (i.e., it can be examined as soon as the blood is visibly dry) rather than letting it dry for several hours [421]. Thin films contain less blood than thick films and are, therefore, less sensitive than thick films; however, they provide ideal morphology for *Plasmodium* species identification and allow for optimal evaluation and differentiation of *Plasmodium* from *Babesia* parasites (although the different *Babesia* species cannot be distinguished from one another by morphology alone). The ordering clinician should notify the laboratory if a highly pathogenic agent such as hemorrhagic fever virus is suspected so that precautions can be taken in preparing and examining the blood films. If applicable, the CDC provides guidelines for inactivating hemorrhagic fever viruses such as Ebola in clinical specimens: https://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimen-management.html).

Both thick and thin films should be screened manually, since automated hematology analyzers may fail to detect *Plasmodium* and *Babesia* species parasites [419]. The slides should first be screened at low power using the 10x objective for identification of microfilariae, followed by examination under oil immersion [414, 419, 420]. The laboratorian should examine a minimum

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of 100 microscopic fields using the 100x objective on the thick and thin films before reporting a specimen as negative. At least 300 additional fields should be examined for patients without previous *Plasmodium* exposure since they may be symptomatic at lower parasite levels. It is important to remember that *Babesia* and *Plasmodium* may at times be indistinguishable on blood films and that both can be transmitted by transfusion so each can occur in atypical clinical settings. Clinical and epidemiologic information must be considered and additional testing may be required.

If parasites are identified and the laboratory does not have expertise for species identification, then a preliminary diagnosis of "*Plasmodium* or *Babesia* parasites" should be made, followed by confirmatory testing at a reference or public health lab. The CDC provides rapid telediagnostic services for this purpose (http://www.cdc.gov/dpdx/contact.html). While awaiting confirmatory testing, the primary laboratory should relay the message to the clinical team that the potentially deadly parasite, *P. falciparum*, cannot be excluded from consideration. Repeat blood samples (3 or more specimens drawn 12 to 24 hours apart, ideally during febrile episodes) are indicated if the initial film is negative, and malaria or babesiosis is strongly suspected [415, 419, 420].

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 is used to guide initial treatment decisions and to follow a patient's response to antimalarial treatment [419].

An alternative to Giemsa-stained blood films for morphologic examination is the Quantitative Buffy Coat (QBC) method, which provides similar sensitivity to blood films [419]. 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 [419]. 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. Malaria RDTs for malaria provide cost effective, rapid alternatives and can be used for screening when blood smear evaluation for blood parasites is not offered locally [420]. These methods are rapid immunochromatographic tests using dipstick, card or cassette formats in which there is a nitrocellulose membrane with bound parasite antigens. The most commonly-used antigens are *Plasmodium* lactate dehydrogenase, *Plasmodium* aldolase, and *P. falciparum* histidine-rich protein-2 (*pf*HRP-2). There are a number of commercially available options, although the BinaxNow® Malaria is currently the only test approved by the FDA and available for clinical use in the United States. Depending on the number of antigens employed, RDTs may detect to the genus level, species level (most commonly *P. falciparum*), or both. The BinaxNow Malaria test detects *pf*HRP-II and *Plasmodium* aldolase, and can therefore detect

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Plasmodium species infection, and *P. falciparum* specifically [418]. Importantly, RDTs are less sensitive than thick blood films and may be falsely negative in cases with low levels of parasitemia (<100 *P. falciparum* parasites/µL) and non-*falciparum* infection. False negative results may also occur due to *P. falciparum* strains with deletions of histidine rich protein 2 (*pf*HRP-2) when using tests that target this protein [422-425]. There can also be false negatives in cases of high parasitemia due to the hook (prozone) effect. In comparison, trained microscopists can detect infections with as little as 5-10 parasites/µL) [419, 420]. The performance characteristics of the commercially available assays vary widely; the WHO provides several useful publications on the performance and selection of available malaria RDTs [418]. Given the lower sensitivity, positive RDTs should be confirmed by examination of thick and thin blood films, ideally within 12 to 24 hours of patient presentation. Blood film examination is also necessary for positive cases to confirm the species present and calculate the degree of parasitemia [420]. In the United States, Canada and Europe, RDTs are primarily used for initial screening in settings where reliable blood films are not be readily available (e.g., night shift, small community laboratories) or when the clinical situation is critical and an immediate diagnosis is required (stat laboratory in the emergency department). When performed, RDT testing should be followed as soon as possible by good quality thick and thin blood film examination. It is important to note that RDTs may remain positive for several days to weeks after successful treatment, since antigens may still be present in the blood. Therefore, blood smears rather than rapid antigen tests should be used to follow patients after therapy [416].

Serology plays little role in diagnosis of acute babesiosis and malaria since antibodies may not appear early in infection and titers may be too low to determine the status of infection. The primary use of antibody detection is for epidemiologic studies and as evidence of previous or relapsing infection. Serologic testing is also used for blood donor screening. Indirect immunofluorescent antibody (IFA) is the most readily available commercial assay for *Babesia*; 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 [416].

Rapid NAATs have recently been developed for malaria and babesiosis and are available from some commercial reference laboratories and the CDC although none are FDA-cleared [416]. These methods offer similar or improved sensitivity to the thick blood film and require no specialized morphologic 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 non-parasitic artifacts. They are also useful for determining *Plasmodium* species. 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

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Plasmodium or *Babesia* parasites, blood films must still be examined to determine the percentage of parasitemia.

It is important to stress that requests for malaria and babesiosis diagnosis should be considered "STAT" and testing performed as rapidly as possible. NAAT assays may be rapid but are usually limited to the reference or public health 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.* In cases where malaria is highly suspected, it may be prudent to start empiric antimalarial therapy while waiting for the laboratory results to return.

American trypanosomiasis/Chagas Disease caused by *Trypanosoma cruzi*

Chagas disease may consist of acute and chronic phases, and the optimal diagnostic method differs by stage. The standard method for diagnosing Chagas disease 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 (the motile blood stage) will be generally present at this time. Motile organisms can be observed in fresh wet preparations of anticoagulated blood or buffy coat, although most U.S. labs do not perform this method. On stained blood films, the presence of a large posterior kinetoplast of *T. cruzi* is the most important morphologic features for differentiating it from *T. brucei*. In comparison, the kinetoplast of *T. brucei* trypomastigotes is much smaller. *T. cruzi* trypomastigotes also commonly assume a "C" shape, but this is not a reliable differentiating feature. These infections can also be differentiated on epidemiologic grounds. Unfortunately, infection is rarely diagnosed in the acute stage since only 1%-2% of infected individuals present with symptoms during this time period [414, 415].

Blood film microscopy is less useful during the chronic stage of infection when rates of parasitemia are very low. In this setting, serology is the test of choice. Diagnosis may also be established during the chronic stage by microscopic examination of myocardial biopsies for detection of the non-motile (amastigote) intracellular form of *T. cruzi*, but this is not a first-line test.

There are a large number of commercially available serologic tests employing whole-parasite or recombinant antigens in ELISA and IFA formats. As there is no single reference standard test, diagnosis of chronic Chagas disease should be based on positive results from at least two different serologic tests, ideally employing different test methodologies [426]. The CDC offers serologic testing with EIA and immunoblot first-line tests. Discordant results are resolved by repeat testing with a second specimen, and if needed, additional testing by a third test such as IFA. PCR testing is also available through the CDC [415]. Positive serology results are considered evidence of active infection and would exclude potential blood/tissue donors who test positive from donating, since the infection has been shown to be transmitted by transfusion and transplantation [415, 426]. Of note, cross-reactivity, particularly with serologic testing using whole-parasite antigens, may be

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seen with leishmaniasis, and additional testing (e.g., with serologic tests using recombinant antigens) may be indicated.

Table 48. Approach to diagnosis of blood and tissue parasitic infections

Disease (organism)	Main Diagnostic Test(s)	Remarks
Amebic meningitis/encephalitis [due primarily to the free-living amebae, *Naegleria fowleri* (PAM), and *Acanthamoeba* spp, and *Balamuthia mandrillaris* (GAE)]	Microscopy of CSF (PAM) or brain tissue (GAE) for rapid antemortem diagnosis (wet prep, stained slides, histopathology). PCR of CSF or brain tissue (available at reference labs and CDC).	PCR is usually more rapid than culture. Specimens for culture should not be refrigerated, as *N. fowleri* will not survive. *Acanthamoeba* spp. and *N. fowleri* will grow in standard culture, but *Balamuthia mandrillaris* requires specialized cell-culture. Stained and unstained tissue slides may be sent to the CDC for identification of amebic trophozoites and/or cysts.
Abdominal angiostrongyliasis (*Angiostrongylus costaricensis*)	Microscopy of histologic sections (intestine, mesentery) for eggs, larvae, and adult worms.	Conventional PCR and sequencing may be performed on tissue sections at the CDC for confirmation of histologic findings.
Eosinophilic meningitis/neural angiostrongyliasis (*Angiostrongylus cantonensis*)	Microscopy of CSF for larvae. CSF PCR at the CDC.	Larvae may also be seen in tissue sections with associated eosinophils and necrosis.
Babesiosis (*Babesia microti, B. divergens, B. duncani, Babesia* sp. MO-1 strain)	Microscopy of Giemsa stained thick and thin blood films	Real time PCR available from CDC and reference labs. Most PCR assays detect *B. microti* only**.** Serology does not distinguish between ongoing and past infection.
Baylisascariasis (Baylisascaris procyonis)	Serology available from the CDC	Larvae may be seen on histopathologic sections of brain and other organ tissue
Cysticercosis (*Taenia solium*)	Serology from the CDC or reference laboratories. Immunoblot testing is highly recommended as a confirmatory test**.** Cross-reactivity may be observed with echinococcosis.	Diagnosis is usually suspected on features of clinical history, physical exam, and neuroimaging studies. Definitive diagnosis is by identification of the cysticercus in involved tissue.

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Echinococcosis (*Echinococcus* spp.)	Serology from the CDC or reference laboratories. Cross-reactivity may be observed with cysticercosis.	Diagnosis is usually suspected on features of clinical history, physical exam, and imaging studies. Definitive diagnosis is by identification of cysts +/- protoscoleces in tissue biopsy or protoscoleces and free hooklets in cyst aspirate. Care must be taken during cyst aspiration to avoid local dissemination and anaphylaxis due to spilled cyst contents. Serology does not differentiate between *E. granulosus* and *E. multilocularis* infection; instead, this is accomplished by imaging and histopathologic examination. Specific *E. multilocularis* serology is available in Europe.
Filariasis due to *Wuchereria, Brugia, Loa loa,* and *Mansonella* species	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 (*Wuchereria bancrofti* and *Brugia malayi*) in blood by the CDC or reference lab	Blood films for most *W. bancrofti* and *B. malayi* strains should be collected between 10pm and 2am when microfilariae are circulating. Collect blood between 10am and 2pm for *Loa loa*. Repeat exams may be necessary due to low parasitemia**.** Serology does not differentiate between filariae. *W. bancrofti* antigen testing can be used on blood collected at any time, but is not available in the United States.
Filariasis, onchocerciasis due to *Onchocerca volvulus*	Microscopy of "skin snip" after incubation in saline at 37°C.	"Skin snips" should be from areas nearby nodules and should be "razor thin" with no visible blood. Histopathologic examination of skin biopsy or resected nodule (onchocercoma) can identify microfilariae and/or adults.
Gnathostomiasis (*Gnathostoma* spp)	Serology is not available in the United States. Contact the CDC for information about laboratories in Thailand and Japan that may provide testing.	Larval forms and immature adults may be seen on histopathologic examination of biopsy specimens.
Leishmaniasis, cutaneous (various *Leishmania* species)	Microscopic exam of Giemsa-stained smears of biopsy touch impressions or aspirate from leading edge of ulcer; culture and PCR are available through the CDC (contact CDC for collection kit prior to collecting biopsy)	Treatment is dependent on species identification (by culture or PCR + sequencing) for disease acquired in South or Central America. Histopathology is less sensitive than smears, culture, and PCR. Serology is not useful for cutaneous disease.
Leishmaniasis, visceral (various	Microscopic exam of Giemsa-stained bone marrow aspirate/biopsy, splenic aspirate;	Positive rK39 serology is reported to be both sensitive and specific for the diagnosis of visceral leishmaniasis in

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Leishmania species)	Culture, PCR, sequencing, and serology is available from the CDC (contact CDC for collection kit prior to collecting biopsy)	various endemic areas of the world. Cross-reactivity with *T. cruzi* may occur.
Malaria (*Plasmodium falciparum, P. ovale, P. vivax, P. malariae, P. knowlesi*)	STAT microscopic examination of Giemsa-stained thick and thin blood films (repeat testing every 12-24 hour for a total of 3 examinations before ruling out malaria); rapid antigen detection tests followed by confirmatory blood films within 12-24 hours	Antigen tests lack sensitivity with low parasitemia and non-*falciparum* malaria and do not differentiate all species. PCR from some reference laboratories will detect and differentiate all species. Calculation of percent parasitemia and species identification (using thick or thin blood films) is required for determining patient management and following response to therapy.
Toxocariasis/Visceral Larva Migrans (*Toxocara canis, T. cati)*	Serology from CDC or reference laboratory	Larvae are only rarely seen in histopathologic sections of biopsies of liver or other infected tissues. Eosinophilic granulomas are usually seen.
Toxoplasmosis (*Toxoplasma gondii*)	Serology (IFA, EIA, ELFA) from CDC or reference lab for detection of IgM and IgG; Positive IgG seen in up to 15 to 40% of U.S. population due to previous exposure. IgG avidity test and serial titers may distinguish between recent and past infection.	Cysts and tachyzoites can be seen in specimens from immunocompromised patients (e.g., bronchoalveolar lavage, brain biopsy); PCR is available from some reference labs. IgG avidity testing is indicated for pregnant women with positive IgM/IgG and is available at the Dr. Jack S. Remington Laboratory for Specialty Diagnostics (formerly the Toxoplasma Serology Laboratory), Palo Alto, CA*. Testing for suspected neonatal cases should also be sent to this laboratory.
Trichinosis (*Trichinella spiralis* and other species)	Serology (EIA) from the CDC or reference laboratory	Encysted larvae can be seen in histopathologic sections of muscle biopsies
Trypanosomiasis, African/African Sleeping Sickness, [*Trypanosoma brucei gambiense* (West African) or *T. b. rhodesiense* (East African)]	Microscopy of Giemsa-stained thick and thin blood films or buffy coat preps. Parasitemia is often low, requiring repeated exams. Aspirates of chancres, lymph nodes, and bone marrow may also be examined. Centrifuged CSF should be examined to evaluate for late-stage disease. There is an infection hazard from live organisms in blood specimens.	Plasma cells with large eosinophilic antibody globules may be seen in CSF and brain biopsy. Card agglutination test for trypanosomiasis (CATT) is available in endemic settings for detection of *T. b. gambiense* infection. Contact the CDC for additional information)
Trypanosomiasis, American	Microscopy of Giemsa stained thick and thin blood films or	Parasitemia is very low in chronic infection; serology is preferred in this

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(Chagas' Disease) due to *Trypanosoma cruzi*	buffy coat preps in acute disease. Serology for chronic infection; available through the CDC and reference laboratories. Positive serology should be confirmed using a second test that employs different antigens. There is an infection hazard from live organism in blood specimens.	setting. IgG antibody may persist for decades and its presence is considered evidence of chronic infection. Molecular testing is available through the CDC.

Notes: "**CDC**" refers to the Division of Parasitic Diseases at the Centers for Disease Control and Prevention, Atlanta GA; https://www.cdc.gov/dpdx/ **"Reference Labs"** refers to any laboratory that performs esoteric testing not usually done in routine hospital labs

*This laboratory is currently the only national source of highly specialized *T. gondii* testing such as IgG avidity testing, and serves as the *T. gondii* reference laboratory for the CDC and FDA.

Abbreviations: CSF – cerebrospinal fluid, EIA – enzyme immunoassay, ELFA - enzyme linked fluorescence antibody, GAE – granulomatous amebic encephalitis, HRP2 – histidine rich protein 2, IFA – immunofluorescence assay, PAM – primary amebic meningoencephalitis

Table 49. Summary of Laboratory Detection Methods for Babesiosis and Malaria Infection [419, 420]

Diagnostic Procedure	Optimum specimen	Transport considerations	Estimated TAT*
Microscopy of Giemsa stained thick and thin blood films with determination of percent parasitemia.	Finger stick or venipuncture (EDTA) blood	Slides should be made from blood within 1 hour. Prolonged exposure to EDTA and to room temperature can alter parasite morphology.	2-4 hours
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 hour for optimal preservation of parasite morphology	2-4 hours
Antigen detection immunochromatographic assay (Rapid Diagnostic Test / RDT).	Drop of blood from finger stick or venipuncture	Test should be performed as soon as possible but blood may be stored at 2°-30° C for up to 3 days for some commercial assays.	15-30 minutes
Serologic detection of antibody to *B. microti* (not generally recommended for malaria diagnosis)	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 hours to preserve antibody and prevent bacterial growth. Avoid use of hyperlipemic or hemolyzed blood.	4-6 hours
NAAT	Typically 1.0 ml venipuncture blood in EDTA tube	Test should be performed as soon as possible but blood may	1-6 hours

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		be transported refrigerated over 48 hours	

* TAT: turn-around-time; transportation time is not included in this estimate

XV. ARTHROPOD BORNE INFECTIONS

Numerous tick genera are associated with transmission of bacterial, parasitic and viral pathogens to humans. This section will focus on tick-, louse- and flea-borne transmission of bacterial and parasitic disease agents; for discussion of arboviruses, the reader is referred to the Virology Section below.

The most common tick-borne diseases in the United States are transmitted by *Ixodes* species ticks, which harbor multiple pathogens including *Borrelia* species (e.g., *Borrelia miyamotoi*, *Borrelia burgdorferi*, *B. mayonii*), *Babesia* spp. and *Anaplasma phagocytophilum* among others, and via *Ornithordoros* ticks, which are primarily associated with transmission of agents of relapsing fever (*Borrelia hermsii, B. turicatae*, etc.). Given the risk of co-transmission, testing for multiple endemic pathogens should be considered by clinicians. Tickborne diseases are also common outside of the United States, including Lyme disease (LD) caused by *B. garinii* or *B. afzelii*, and tick-borne rickettsial diseases, such as African tick-bite fever (ATBF) and Mediterranean spotted fever (MSF), which are caused by *Rickettsia akari* and *R. conorii*, respectively (see below) [427]. As a result, travel and exposure history is essential for clinicians to be cognizant of in order to order the correct diagnostic assays.

LD, or Lyme borreliosis is caused by members of the *B. burgdorferi i*sensu lato complex, with 18 genomic species either confirmed or considered to potentially cause human disease, among which four, including *B. burgdorferi, B. mayonii, B. garinii* and *B. afzelii*, are most commonly identified [428]. LD is a multisystem disease that can affect the skin, nervous system, the joints, and heart, and is the most frequently reported tick-borne disease in the northern hemisphere [429]. Most commonly, early localized LD is diagnosed on clinical grounds, including the presence of erythema migrans in approximately 70% to 80% of patients. Erythema migrans (an expanding rash) is largely considered pathognomonic for Lyme borreliosis, however other conditions can mimic this dermatologic presentation (e.g., southern tick-associated rash illness [STARI], cellulitis, etc.).Diagnostic testing for LD in patients who present with a characteristic EM rash, alongside an appropriate exposure history, is contraindicated, as antibodies to *B. burgdorferi* are likely not yet detectable, leading to a low negative predictive value and increased risk for undertreatment. Culture of tissue biopsies is no longer routinely performed due to the need for special culture media, variable sensitivity (60%-90%) and long turnaround time [430].

While nucleic acid amplification testing (NAAT) for LD-associated *Borrelia* species is available through multiple reference laboratories, performance of this testing on whole blood, other blood fractions or CSF for detection of LD or neuroinvasive LD is not recommended due to low sensitivity (range: 18% - 22.5%) in these specimen sources [431, 432]. A notable exception to this is the low sensitivity of NAAT for detection of LD is *B. mayonii*; this newly described agent of

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LD is associated with a higher level of spirochetemia and given the lack of serologic assays able to detect specific antibodies to this species(i.e., current serologic testing for Lyme disease based on *B. burgdorferi* antigens will be negative in patients with *B. mayonii* infection), NAAT of whole blood is recommended for detection of *B. mayonii* [428]. Currently, *B. mayonii* infections have only been documented in Minnesota and Wisconsin; therefore diagnostic testing by NAAT for this species should only be considered in patients with tick exposure in these regions and who present with symptomatic disease. NAAT for *B. burgdorferi* is most sensitive and has the highest clinical yield when performed on erythema migrans tissue biopsies for assessment of suspected or atypical-appearing lesions (median sensitivity 68%) or synovial fluid (median sensitivity 77.5% for suspected LD arthritis cases [433]. Of note, for patients in whom the EM lesion has a characteristic 'bulls-eye' appearance and who recall recent tick exposure, a diagnosis of LD solely based on the EM appearance is sufficient as this is a pathognomonic sign of infection [434]. In patients presenting with suspected joint infection due to *B. burgdorferi*, serologic testing for LD will be positive in nearly all patients as this is a later manifestation of disease, which in many cases limits the need for additional molecular testing on synovial fluid [433]. For patients with suspected neuroinvasive LD, current recommendations include serologic assessment for LD antibodies in blood and CSF, using an antibody index assay [435].

Serologic testing using a two-tiered testing algorithm (TTTA) remains the testing methodology of choice for both early disseminated and late stages of LD. There are currently two TTTA approved by the CDC, either the standard (STTTA) or modified (MTTTA) algorithms. Both start with an initial EIA (or less frequently IFA) screen for antibodies to LD-associated *Borrelia* species. Samples reactive by the first-tier screening assay are reflexed for supplemental testing by IgM- and/or IgG- Western or immunoblots for the STTTA or, by supplemental IgM and/or IgG EIAs for the MTTTA. One of the key advantages of the MTTTA is the improved sensitivity for antibody detection in patients with early LD as compared to testing of these same patients using the STTTA (74% vs. 41%) [436]. Importantly, a two-tiered approach is required, regardless of which algorithm is used, in order to maintain optimal specificity.

While second tier testing as part of the MTTTA involves qualitative 'positive' or 'negative' results, immunoblot test result interpretations as part of the STTTA are more complex. For *B. burgdorferi*-specific IgG and IgM immunoblots to be considered positive, the presence of at least 5 out of a possible 10 diagnostic IgG bands and at least 2 out of a possible 3 IgM bands must be observed, ideally using optical densitometry measurement platforms rather than interpretation by the naked eye [437]. Importantly, the IgM blot is not clinically meaningful in patients who present 30 days or longer post symptom onset due to high rates of false positivity, and, therefore, is not recommended. Additionally, seropositivity for both IgM- and IgG-class antibodies to LD-associated *Borrelia* species may persist for months to years (>10-15 years) following resolution of the infection [438, 439]. Since positivity by either TTTA may reflect remote exposure rather than current infection, it is recommended that only symptomatic patients with an appropriate exposure history be tested for LD. Finally, multiple LD 'specialty' laboratories have emerged in recent

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years, claiming expertise in tick-borne disease diagnosis and offering LD diagnostic assays with improved sensitivity [440]. These laboratories may not be CLIA-approved and offer LD diagnostic assays using methods and interpretive criteria for which validation data has neither been made publicly available nor been vetted by high quality peer-review. Submission of patient specimens to such laboratories is not recommended.

Classical relapsing fever, caused by *B. hermsii, B. parkeri, B. mazzottii* or *B. turicatae* which are transmitted by the bites of soft (argasid) ticks, burden residents and travelers to multiple states in the western and southwestern part of North America, although sporadic cases occur in south-central states as well. Louse borne relapsing fever (LBRF), primarily caused by *Borrelia recurrentis*, is endemic to tropical countries or may become epidemic in refugee camps [441]; travelers would be the only patients that might present with LBRF and their diagnosis would be similar to that for tick borne relapsing fever. Relapsing fever presents as recurrent fevers of several days duration, terminating with crisis and resuming after a few days. Febrile episodes are marked by the presence of large numbers of spirochetes in the peripheral blood. Relapsing fever-like borreliae (*B. miyamotoi*) transmitted by *Ixodes* species ticks cause fever that has a less characteristic presentation and may be confused with human granulocytic anaplasmosis; spirochetes are sparse in peripheral blood but are usually detectable by NAAT. Recent data suggests that both acute and convalescent sera from patients with *Borrelia miyamotoi* infection (BMI) are frequently reactive by first tier serologic assays for Lyme disease and convalescent sera may be positive of *B. burgdorferi*-specific IgM blots [442]. Despite this, testing for BMI using *B. burgdorferi* serologic assays is not recommended. Given the high spirochetemia during acute disease however, the preferred diagnostic approach is assessment for spirochetes in peripheral blood smears, or alternatively by NAAT where available.

In the United States, tick-transmitted rickettsial diseases include Rocky Mountain spotted fever (RMSF) caused by *Rickettsia rickettsii*; "mild" RMSF (*R. parkeri* and other spotted fever group *Rickettsia* spp), human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), human monocytic ehrlichiosis (*Ehrlichia chaffeensis*), and other ehrlichioses caused by *Ehrlichia ewingii*, and *E. muris eauclairensis* [443, 444]. Although clinically similar, these diseases are epidemiologically and etiologically distinct illnesses. Endemic typhus and flea borne typhus (*R. typhi* and *R. felis*, respectively) may also infect people in the U.S., mainly in warmer sites where fleas are common throughout the year. Rare epidemic typhus (*R. prowazekii*) cases have been recorded in the U.S. from contact with flying squirrels or their nests. Rickettsialpox (*R. akari*), comprising a mild febrile disease with rash and eschar, is maintained by mouse mites in many large urban areas. The diagnosis of patients with these infections is challenging early in the course of their clinical infection since signs and symptoms are often nonspecific or mimic benign viral illnesses. Rash is usually present in most acute rickettsiosis, but skin color may prevent its recognition. The likelihood of severe morbidity or mortality with delaying treatment for RMSF means that patients should be presumptively treated without waiting for laboratory confirmation, which rests mainly on seroconversion. Notably, serologic testing for *Rickettsia* species is not

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valuable for an immediate diagnosis and is frequently primarily available through public health and select reference laboratories. Ideally, if serologic testing is used, an acute serum sample obtained within 7 days of symptom onset and a convalescent serum obtained at least 21 days later, should be submitted for testing. The sensitivity of serologic assays for tickborne rickettsial infection ranges from 94% to 100% after 14 days of symptoms [444]. Of not however, early antibiotic treatment can blunt the antibody response and antibody levels may fall quickly during the months after exposure. Due to the significant risk for morbidity however, antibiotic treatment should *not* be postponed while awaiting test results.

In contrast diagnosis of anaplasmosis or ehrlichiosis relies on NAAT during the acute stage of disease (<10 days post-symptom onset) and on seroconversion or elevated IgG antibody titers (ie, >1:128) for patients presenting beyond this timeframe. Assessment for *A. phagocytophilum* or *Ehrlichia* spp. morulae in lymphocytes is not recommended due to low sensitivity as compared to NAAT (20%-75% versus >95%) during the acute stage of disease [445, 446]. Serologic testing for *A. phagocytophilum* is associated with a specificity of 83-100%, with cross-reactivity occurring in patients infected with *Ehrlichia* species, *Rickettsia rickettsiae,* and *Coxiella burnetti* among others. A newly discovered *Ehrlichia* species, *Ehrlichia muris eauclairensis* was reported to cause ehrlichiosis in Minnesota and Wisconsin and may likely be detected by serology due to cross-reactivity [443]

In addition to borreliosis and rickettsial diseases, babesiosis and tularemia are also transmitted by ticks in the U.S. With the exception of babesiosis, which may comprise as much as a third as many cases as Lyme borreliosis in some sites, these other tick borne infections occur much less frequently (a tenth as common as Lyme borreliosis). Similar to diagnostic testing recommendation for *Anaplasma* and *Ehrlichia,* diagnosis of acute *Babesia* infections should rely on molecular means, whereas patients presenting beyond 7days post-symptom onset should be evaluated using IgG serologic testing (see section on Blood and Tissue Parasite Infections). Diagnosis of *F. tularensis* relies on culture (lymph node aspirates, rarely from whole blood), with clinicians encouraged to notify the laboratory if tularensis is suspected due to the risk of laboratory acquired infections. Serologic testing relies on microagglutination test (IgM and IgG) with most patients seroconverting 2-3 weeks after onset of illness.

Body lice may transmit *Bartonella quintana,* the agent of trench fever, while fleas are associated with transmission of *Bartonella henselae*, the cause of cat scratch disease. Transmission may occur through bites from these arthropods, but a more likely mode of exposure is to the infectious louse or flea excreta. Bartonelloses may present as acute febrile disease, with or without lymphadenopathy. These Gram-negative bacteria are fastidious and slow growing (up to 4 weeks of incubation), requiring hemin and a humidified carbon dioxide atmosphere. If lymphadenopathy is present, aspirates may be cultured; whole blood needs to be lysed for effective cultivation, although these pathogens are rarely recovered in blood culture; as a result of these challenges, culture for *Bartonella* species is not routinely recommended. NAATs have been developed at a number of reference laboratories. Sensitivity is higher for tissue samples as compared to blood

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or blood fraction specimens, but there may be low bacterial copy number or tissue PCR inhibitors that influence the sensitivity of the test; a negative result does not rule out Bartonellosis. Indirect immunofluorescent antibody testing remains the reference method for confirmation of Bartonellosis, particularly if seroconversion is documented. There is significant IgG cross reactivity between the *Bartonella* species, thus specific identification of the infecting species may not be possible without culture or NAAT. Patient management does not necessarily rely on specific identification of the infecting *Bartonella* sp., indeed, risk factors are often sufficient for presumptive identification (a history of homelessness or IV drug abuse would be consistent with *B. quintana* infection; a veterinarian would be likely to be exposed to *B. henselae*). Endocarditis should be ruled out for those with prolonged fever and elevated IgG titers.

Given that many of the vector-borne pathogens discussed above are infrequently encountered in clinical specimens, alongside the limited availability of FDA-cleared or approved assays, many clinical microbiology laboratories refer testing to commercial reference laboratories. Although local hospitals have the ability to perform blood smears for detection of relapsing fever, ehrlichiosis, anaplasmosis, and babesiosis, it should be noted that a negative smear result does not necessarily rule out these tick-borne infections due to the low and variable sensitivity of a peripheral blood smear examination. However, when laboratory staff is experienced, blood smears may be rapidly done because the presence of spirochetes or intraerythrocytic (malaria, babesia) or intraleukocytic (*Anaplasma*, *Ehrlichia*) inclusions is definitive. As with most infections, paired acute (at presentation) and convalescent (2-3 weeks post-presentation) serologic testing of patients suspected of having a vector-borne disease, provide the best probability of confirming a diagnosis. Metagenomic next generation sequencing (mNGS) from blood, although still not widely available or routinely utilized, does offer a potential future improvement in the detection of these pathogens given that this is a symptom-agnostic diagnostic approach. However, limitations relative to assay sensitivity will remain, similar to current NAATs, and will largely depend on when samples were collected relative to disease onset. Overall, given the variability of available testing and the importance of ordering the right test at the right time relative to patient presentation, clinicians are encouraged to check with their reference laboratory regarding test availability, the optimal testing approach, specimen source, and turn-around time.

Finally, while laboratory identification of arthropods submitted by patients can provide some information with respect to exposure risk, testing of these arthropods for the presence of infectious agents has no clinical value as presence of the organism in the arthropod does not confirm transmission to the patient. Instead, only symptomatic patients should be tested for specific vector-borne infections, guided by clinical presentation, duration of symptoms, and exposure history.

Key points for the laboratory diagnosis of arthropod-borne infections:

- Arthropod borne diseases may be difficult to diagnose because signs and symptoms are generally non-specific early in infection, including fever, chills, aches, pains, and rashes

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- Patient residence, travel history, recent exposure, time of year and potential for tick bite are important
- Serology remains the best tool for confirming the diagnosis of Lyme disease. The exception to this is for patients with EM, which is sufficient for a clinician-based diagnosis.
- NAATs are the preferred diagnostic modality for acute infection with *Anaplasma, Borrelia miyamotoi, Babesia* species and *Ehrlichia* species. *Babesia* may also be a microscopic diagnosis where available.
- Consultation with the microbiology laboratory is normally required to determine the specimens accepted, the available diagnostic assays, the location of the testing laboratory, and the turnaround time for results

Table 50. Laboratory Diagnosis of Tickborne Infections

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues
Bacteria			
Relapsing fever borreliae *Borrelia hermsii* (western USA) *Borrelia parkeri* (western USA) *Borrelia turicae* (southwestern USA) *Borrelia mazzottii* (southern USA)	Primary test[1]: 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
	Others Tests[2] NAAT Serologic testing	Serum, blood, body fluids Serum	Clot tube for serum; sterile tube or citrate tube for body fluids, RT, within 2-4 h
Borrelia burgdorferi sensu lato complex (Lyme borreliosis)[3] *Borrelia burgdorferi* (USA) *Borrelia mayonii* (USA) *Borrelia garinii* (Europe, Asia) *Borrelia afzelii* (Europe, Asia)	Early, localized Lyme disease with EM[4] Testing not routinely recommended (See NAAT below)	Not applicable	

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	Early, disseminated: If EM or multiple EM rash absent (weeks through months after tick bite) or late (months through years after tick bite) in untreated patients: Primary test: Two-tier testing (acute- and convalescent-phase sera optimal) = EIA antibody screening. If EIA result is positive or equivocal, supplemental IgM/IgG immunoblots or EIAs are required NOTE: Immunoblot or supplemental EIAs should NOT be performed unless an initial EIA is reported as positive or equivocal.	Serum	Clot tube, RT, ≤ 2 h
	Early Lyme Neuroborreliosis: Two-tiered testing algorithm Late Lyme Neuroborreliosis CSF/Serum Antibody Index	Serum Paired serum and CSF, collected within 24 hrs	Clot tube, RT, ≤ 2 h Clot tube for serum, sterile tube for CSF, RT, ≤1 h
	NAAT	Biopsy specimens of infected skin, synovial fluid or tissue, etc.	Transport on ice; ≤1 h. If DNA not extracted shortly after collection, store frozen at -70□C.
Borrelia miyamotoi (*B. miyamotoi* infection, BMI, hard tick borne relapsing fever)	Primary test for acute infection: NAAT	Blood	Transport on ice; ≤1 h. If DNA not extracted shortly after collection, store frozen at -70□C.
	Serology: EIA for detection of antibodies to recombinant GlpQ antigen	Serum	Clot tube, RT, <2h

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Anaplasma phagocytophilum (human granulocytotropic anaplasmosis)	Primary test for acute infection: NAAT	Blood	EDTA anticoagulant tube Transport on ice; ≤1 h
	Alternative Primary Test[5] (if experienced technologists available/NAAT unavailable): Wright or Giemsa stain of peripheral blood or buffy coat leukocytes during week first week of infection.	Blood	EDTA or citrate tube, RT, ≤1 h
	Serology: Acute and convalescent IFA titers for IgG-class antibodies to *A. phagocytophilum* antibodies NOTE: Not recommended for acute infection	Serum	Clot tube, RT, ≤2 h
	Immunohistochemical staining of *Anaplasma* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)	Formalin container, RT, ≤2 h
Ehrlichia chaffeensis (human monocytotropic ehrlichiosis) *Ehrlichia muris* *Ehrlichia ewingii*	Primary test for acute infection: NAAT NOTE: Only definitive diagnostic assay for *E. ewingii*)	Whole blood	Heparin or EDTA anticoagulant tube Transport on ice; ≤ 1 h If DNA not extracted shortly after collection, store frozen.
	Wright or Giemsa stain of peripheral blood or buffy coat leukocytes smear during first week of infection.[5]	Blood	EDTA anticoagulant tube, RT, ≤1 h
	Serology: acute and convalescent IFA titers for *Ehrlichia* IgG-class antibodies NOTE: Not recommended for acute infection	Serum	Clot tube, RT, ≤2 h

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	Immunohistochemical staining of *Ehrlichia* antigens in formalin-fixed, paraffin-embedded specimens	Bone marrow biopsies or autopsy tissues (spleen, lymph nodes, liver and lung)	Formalin container, RT, ≤2 h
Rickettsia rickettsii (Rocky Mountain spotted fever) *Other spotted fever group Rickettsia spp(mild spotted fever)* *R. typhi (murine typhus)* *R. akari (rickettsialpox)* *R. prowazekii (epidemic typhus)*	Serology: acute and convalescent IFA for *Rickettsia sp*. IgM and IgG antibodies	Serum	Clot tube, RT, ≤2 h
	NAAT	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart, and brain)	Sterile container Transport on ice; ≤1 h If DNA not extracted shortly after collection, store frozen.
	Immunohistochemical staining of spotted fever group rickettsiae antigens (up to first 24 h after antibiotic therapy initiated) in formalin-fixed, paraffin-embedded specimens	Skin biopsy (preferably a maculopapule containing petechiae or the margin of an eschar) or autopsy tissues (liver, spleen, lung, heart and brain)	Formalin container, RT, ≤2 h
Protozoa			
Babesia microti *Babesia sp.*	Primary Test: Giemsa, Wright's, Wright-Giemsa stains of peripheral thin and thick blood smears (Giemsa preferred)	Whole blood Second choice EDTA vacutainer tube	For whole blood, prepare smears immediately RT, ≤30 min
	Primary Test for acute infection: NAAT	Blood	EDTA anticoagulant tube, RT, ≤1 h
	Serology[6]: acute and convalescent IFA titers for *Babesia* IgG-class antibodies NOTE: Not recommended for acute infection.	Serum	Clot tube, RT, ≤2 h

[1]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.

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[2]Culture is infrequently performed outside of public health or research laboratories. 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 [438].
[3]Serologic assays used in North America are designed to detect antibodies to *B. burgdorferi* sensu stricto. These assays, particularly the blots, are insensitive for detection of *B. garinii, B. afzelii,* or *B. mayonii* antibodies. Immunoblots for detection of antibodies to *B. garinii* or *B. afzelii* are available at select commercial reference laboratories.
[4]Erythema migrans (EM) is the only manifestation of Lyme disease in the U.S. that is sufficiently distinctive to allow clinical diagnosis in the absence of laboratory confirmation.
[5]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.
[6]Currently available serologic assays are designed specifically for *B. microti* and may not detect antibodies to other *Babesia* spp (e.g., *B. duncani, B. divergens,* etc.).

XVI. VIRAL SYNDROMES

This section will review commonly encountered viral infections in the U.S., realizing there are a myriad of viruses associated with human disease. Clinical microbiology laboratory tests that are commonly used to establish a diagnosis of viral infections are outlined below. This section will review recommended testing for the following categories of viral infection: 1) Common childhood viral infections; 2) herpesviruses, including cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus-6 (HHV-6), herpes simplex virus (HSV), and varicella-zoster virus (VZV); 3) hepatitis viruses (i.e., hepatitis A-E viruses); 4) polyomaviruses, including JC virus and BK virus; 5) respiratory viruses, such as adenovirus, coronaviruses, influenza virus, and respiratory syncytial virus (RSV); 6) retroviruses; 7) vaccine-preventable diseases, including measles, mumps and rubella; and 8) zoonotic viruses, including arboviruses (e.g., dengue, West Nile virus, Zika) and those transmitted primarily through an animal vector (e.g., lymphocytic choriomeningitis virus, rabies). Not all clinical microbiology laboratories provide the comprehensive services outlined in the tables below, especially in the case of serologic and molecular tests. When the recommended testing is not available in a local laboratory, it can often be referred to a reference or public health laboratory, although this approach may yield a delay in obtaining results.

Though an increasing number of molecular tests for infectious agents are gaining FDA clearance, many molecular assays for viral pathogens are laboratory developed tests (LDTs), offered by Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories. Although LDTs require validation according to CLIA requirements prior to clinical use, performance may vary between laboratories. Throughout this section, the acronym NAAT (nucleic acid amplification test) generally refers to polymerase chain reaction (PCR) or reverse transcriptase real-time PCR. Other specific techniques may be substituted with appropriate validation.

While the results of molecular assays offer strong laboratory evidence for the presence or absence of a viral agent, serologic tests may not be as conclusive. Notably, detection of IgM-class

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antibodies against a variety of viral agents may be associated with false-positive results. Therefore, if the pretest probability of acute infection is low to moderate, it is good practice to measure IgG (or total IgG and IgM) antibodies at the time of presentation (acute phase) and two to three weeks later (convalescent phase) to assess for seroconversion, or if possible and depending on the assay format, to demonstrate a four-fold or greater rise in antibody titers.

Key points for the laboratory diagnosis of viral syndromes:

- Viral syndromes should be considered based on the patient's age, immune status, exposure and vaccination history, and many other variables
- Samples should be obtained and tested for the most likely agents, with residual specimen being stored (preferably frozen) in the laboratory in case additional testing is necessary. Typically, it is not cost-effective to test initial samples broadly for numerous viruses; however, multiplex viral testing may be beneficial in immunocompromised hosts or those who are severely ill.
- Sample collection and handling are essential components of obtaining a reliable viral test result; consult the microbiology laboratory to determine which specimens should be obtained and how to transport them to the laboratory.
- Many laboratories will not have broad virologic testing capabilities, requiring specimens to be referred externally and resulting in longer turnaround times for results.
- Antibody cross-reactivity among some closely related viral agents may result in non-specific serologic results.
- Tests for immunity, previous viral infection (e.g., for tissue donors), and new infections may have different assay formats, even when the same virus is being evaluated.

Common (non-herpesvirus) childhood viral infections

Enterovirus and Parechovirus

Enteroviruses are a large group of viral pathogens that may cause disease ranging from mild respiratory infection to paralysis or severe central nervous system infection. NAAT of CSF is more sensitive than viral culture for the diagnosis of enteroviral central nervous system infection (Table 51). Plasma or serum NAAT is useful for diagnosis of sepsis syndrome in a newborn due to enterovirus, but testing is less reliable beyond the newborn period. In the right clinical scenario, detection of enterovirus from throat or stool specimens may provide circumstantial evidence of CNS infection; however, if this is performed, it should be accompanied by NAAT testing of CSF. Cases of severe acute respiratory illness in young children may be due to enterovirus D68, which has been associated with acute flaccid myelitis (AFM) in some cases. Initial testing for enterovirus D68 may include molecular testing of nasal, nasopharyngeal or throat swabs. If the patient develops any signs of AFM or central nervous system involvement, testing of CSF is recommended. Currently, most routine molecular tests will detect D68; however, they will not yet differentiate infection caused by D68 from other serotypes. A positive result can be investigated

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further at public health laboratories by sequencing or serotyping to determine if the cause of infection is D68.

Serologic evaluation for enteroviruses requires assessment of acute and convalescent titers, due to the high (e.g., >75% seropositivity by 10 years of age for coxsackievirus A6) seroprevalence in the population [447]. Therefore, serology is typically not useful in clinical practice, except for determining whether a patient with myocarditis has had exposure to enteroviruses (e.g., coxsackie B virus).

Parechoviruses can result in clinical disease similar to that caused by enteroviruses but are classified as a different genus and require a specific NAAT for detection (laboratory-developed only, except for one current multiplex assay that is an FDA-cleared tests).

Table 51. Laboratory Diagnosis of Enterovirus and Parechovirus Infections

Diagnostic Procedures	Optimal Specimen	Transport Issues
NAAT	Cerebrospinal fluid[1]	Sterile, preservative-free tube, RT, ≤24 h
	Plasma[2]	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum[2]	SST tube, RT ≤2 h
	Urine	Sterile, preservative-free container, RT, ≤24 h
Culture	Plasma[2]	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Stool	Sterile, preservative-free container, RT, ≤24 h
	Throat swab	Sterile, preservative-free container or viral transport medium, RT, ≤2r h

[1] A commercial FDA-cleared product is available for rapid PCR testing for enteroviruses in CSF.
[2] Whole blood is a less reliable source for detection by culture or NAAT methods.
PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Parvovirus B19

Parvovirus B19 is associated with a variety of clinical syndromes including erythema infectiosum (i.e., 'slapped-cheek' rash or 'gloves-and-socks' syndrome) or arthralgia/arthritis in immunocompetent individuals, transient aplastic crisis in patients with hemoglobinopathies or who are otherwise immunosuppressed, and congenital infection and possibly fetal death (e.g., hydrops fetalis). Disease is often bi-phasic beginning as a self-resolving, non-specific febrile illness, followed by onset of rash and/or arthralgia approximately one week later. Importantly, the classic rash is immunologically mediated, as its appearance corresponds with development of an IgM antibody response to the virus.

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Serologic testing for the presence of IgM- and/or IgG-class antibodies to parvovirus B19 is the recommended diagnostic testing method for evaluation of a parvovirus B19 infection (Table 52). IgM-class antibodies to the virus are detectable within 10-12 days post infection, with IgG detectable by 2 weeks [448-450]. Notably, approximately 90% of patients presenting with erythema infectiosum have detectable IgM antibodies to parvovirus B19 at the time of presentation [450]. Antibodies to parvovirus B19 reach peak titers within one month, and while the presence of IgM-class antibodies suggests recent infection, they can persist for months. The presence of IgG antibodies alone is indicative of past exposure; these may remain detectable for life and are thought to provide lasting immunity to re-infection. Serologic testing for parvovirus B19 remains the recommended methodology for evaluation of pregnant women with possible exposure or infection; positive results for both IgM and IgG antibodies to parvovirus B19 suggest infection within the last three months and a possible risk of infection to the fetus. Importantly, serologic tests may be negative in an immunocompromised host, despite prior exposure to the virus.

Parvovirus B19 NAATs may provide improved sensitivity over serologic methods in patients presenting with transient aplastic crisis or chronic anemia. Despite the lack of FDA-cleared molecular assays for parvovirus B19, NAAT is the preferred non-invasive technique for laboratory diagnosis of parvovirus B19-related anemia in immunosuppressed individuals, including solid organ transplant recipients. An important caveat regarding NAAT for diagnosis of parvovirus B19-related anemia is that parvovirus B19 DNA has been anecdotally detected for extended periods in serum, even in healthy individuals [451]. The presence of giant pronormoblasts in bone marrow on histopathologic or cytologic studies is suggestive of parvovirus B19 infection, although such cells are not always detected.

Table 52. Laboratory Diagnosis of Parvovirus (Erythrovirus) B19 Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum	SST tube, RT, ≤2 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h
Histopathology or Cytology	Bone marrow	Sterile container, RT;, ≤24 h; Formalin-filled container, RT
Serology	Serum	Clot or SST tube, RT, ≤2 h

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Herpesviruses

Cytomegalovirus

Cytomegalovirus (CMV) is a member of the Herpesviridae family and causes acute and latent infection. Infection with CMV is very common, resulting in mild or asymptomatic disease in most immunocompetent individuals. However, CMV is a significant cause of morbidity and mortality among immunocompromised hosts, especially transplant recipients. Serologic testing for CMV-

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specific antibodies is typically limited to pre-transplant screening of the donor and recipient (Table 53). This is usually accomplished by testing for anti-CMV IgG-class antibodies, which when present, indicate past exposure to CMV. CMV exposure and seropositivity increases with age, with approximately 36% seroprevalence among children ages 6-11 years and increasing to over 90% among adults age 80 years and older [452]. The utility of testing for IgM-class antibodies is more limited and may serve as an adjunct in the diagnosis of recent CMV infection; however, false-positive CMV IgM results may occur in patients infected with EBV or with immune disorders.

In recipients of solid organ or peripheral blood stem cell transplants, monitoring CMV viral loads by a quantitative NAAT is used to diagnose CMV-associated signs and symptoms, to guide preemptive treatment, and to monitor response to antiviral therapy. For laboratories using LDTs, Standard Reference Material (SRM) is available from the National Institute of Standards and Technology (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 (e.g., copies/microliter). However, multiple FDA-approved assays (Abbott RealTi*me* CMV and Alinity m CMV, Abbott Molecular, Inc.; *artus*® CMV RGQ MDx Kit, Qiagen, Inc.; Cobas® AmpliPrep/Cobas® TaqMan® CMV Test and Cobas® CMV, Roche Molecular Systems, Inc.; Aptima® CMV Quant, Hologic) are now available that are calibrated against the WHO standard and allows for normalization of results to international units (IU)/mL. Conversion of copies/mL to IU/mL using the World Health Organization Standard (or a WHO traceable standard) allows for laboratory-to-laboratory comparison of results. Qualitative detection of CMV DNA in a variety of specimen types can also assist in the diagnosis of disease.

Cytomegalovirus was cultured from peripheral blood mononuclear cells (and other clinical specimens) in the past as a routine diagnostic method (i.e., buffy coat). However, isolation is labor-intensive and can take up to 14 days. The turnaround time can be reduced to 1 to 2 days with the use of the shell vial assay. In addition to a long turnaround time, culture-based assays have poor sensitivity for the recovery of CMV. Because the viral load is typically high and CMV is shed in the urine of newborns, urine culture for CMV continues to be used at some institutions for the diagnosis of congenital CMV infection. The diagnosis of congenital CMV requires either recovery of the virus, or the detection of CMV DNA in urine, saliva, blood or CSF during the first 3 weeks of life.

Cytomegalovirus antigens can be demonstrated by immunohistochemical or *in situ* hybridization tests of formalin-fixed, paraffin-embedded tissues.

Among immunocompromised patients with CMV infection, the potential exists for the emergence of resistance to antiviral agents. A variety of assays can be used to assess antiviral resistance, most commonly by sequencing of the *UL97* (phosphotransferase gene) and *UL54* (DNA polymerase gene) genes. Sequencing-based assays are performed on DNA amplified directly from clinical

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specimens, provided they contain sufficient quantity of CMV DNA. Ganciclovir resistance most commonly emerges due to point mutations or deletions in *UL97* (with foscarnet and cidofovir unaffected) with mutations at three codons with codons *460, 594, 595* being most common. *UL54* point mutations or deletions occur less frequently. If *UL54* mutations are selected by ganciclovir or cidofovir, there is typically cross-resistance to both ganciclovir and cidofovir but not foscarnet. However, if mutations are selected by foscarnet, there is usually no cross-resistance to ganciclovir or cidofovir.

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

Table 53. Laboratory Diagnosis of Cytomegalovirus (CMV) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT, qualitative	Body fluids Cerebrospinal fluid Respiratory specimens Tissue Urine	Sterile, preservative-free container, RT, ≤24 h
NAAT, quantitative (viral load)	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h
Serology	Serum	Clot or SST tube, RT, ≤2 h
	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
Culture	Urine	Sterile, preservative-free container, RT, ≤24 h

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube

Epstein-Barr virus

Epstein-Barr virus (EBV) is a cause of mononucleosis among immunocompetent individuals and lymphoproliferative disease in immunocompromised patients. An elevated white blood cell count with an increased percentage of atypical lymphocytes is common in EBV-associated mononucleosis. Heterophile antibodies usually become detectable 6 to 10 days following symptom onset, increase through the second or third week of the illness and, thereafter, gradually decline over a year or longer. False-positive heterophile antibody results may be observed in patients with autoimmune disorders, leukemia, pancreatic carcinoma, viral hepatitis, or CMV infection. False-negative results are obtained in approximately 10% of patients and are especially common in children younger than 4 years.

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When the results of rapid Monospot or heterophile testing are negative, additional laboratory testing (Table 54) may be considered to differentiate EBV infection from a mononucleosis-like illness caused by CMV, HIV, or *Toxoplasma gondii*. In this situation, EBV-specific antibody testing for IgG- and IgM-class antibodies to the viral capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA) is recommended. The presence of VCA IgM (with or without VCA IgG) antibodies in the absence of IgG-antibodies to EBNA suggests recent, primary infection with EBV. The presence of anti-EBNA IgG antibodies indicates that infection occurred at least 6 to 12 weeks prior, and therefore, is suggestive of a past (remote) infection with EBV. IgG-class antibodies to EBNA generally develop two to three months after primary infection and are detectable for life. Over 90% of the adult population has IgG-class antibodies to VCA and EBNA antigens, although approximately 5% to 10% of patients who have been infected with EBV fail to develop antibodies to EBNA. Nucleic acid amplification tests, either qualitative or quantitative, are not recommended for routine diagnosis of EBV-associated disease (e.g., mononucleosis) in the immunocompetent patient population.

EBV is associated with lymphoproliferative disease in patients with congenital or acquired immunodeficiency, including patients with severe combined immunodeficiency, recipients of organ or peripheral blood stem cell transplants, and patients infected with HIV. An increase in the EBV viral load in peripheral blood or plasma, as measured by a quantitative NAAT, may occur in patients before the development of EBV-associated lymphoproliferative disease. Viral loads should be measured no more frequently than once per week, and these levels typically decrease with effective therapy. A difference in the viral load of $\geq 0.5 \log_{10}$ between samples, preferably evaluated by the same assay, is typically required to demonstrate a significant change. Conversion of EBV copies/mL to IU/mL using the World Health Organization Standard (or a WHO traceable standard) allows for laboratory-to-laboratory comparison of results. An FDA-approved test (Abbott m EBV, Abbott Molecular, Inc.; Cobas® EBV, Roche Molecular Systems, Inc.) is now available for detection and quantification of EBV in plasma samples. Tissues from patients with EBV-associated lymphoproliferative disease may show monoclonal, oligoclonal, or polyclonal lesions. The diagnosis of EBV-associated lymphoproliferative disease (e.g., post-transplant lymphoproliferative disorder [PTLD]) requires multiple tests, including quantitative NAAT, radiology (e.g., positron emission tomography [PET] scan), and detection of EBV DNA, RNA or protein in biopsy tissue.

NAATs may be used to detect EBV DNA in cerebrospinal fluid (CSF) of patients with acquired immunodeficiency syndrome-related central nervous system lymphoma. However, EBV DNA may also be present in the CSF of patients with other abnormalities (e.g., central nervous system toxoplasmosis, pyogenic brain abscesses), and therefore, positivity is non-diagnostic. Detection of EBV-specific antibodies in CSF may indicate central nervous system infection; however, it may also be observed if the CSF fluid becomes contaminated with blood during collection, or if there is transfer of antibodies across the blood-brain barrier. Calculation of the CSF-to-serum antibody index may be helpful, but this type of testing is not performed in most clinical laboratories.

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Table 54. Laboratory Diagnosis of Epstein-Barr Virus (EBV) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology (include heterophile antibody test or Monospot)	Serum	Clot or SST tube, RT, ≤2 h
NAAT, qualitative	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
NAAT, quantitative (viral load)	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Whole blood, peripheral blood lymphocytes	EDTA or citrate tube, RT, ≤2 h

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Human herpes virus-6

Human herpes virus-6 (HHV-6) causes roseola infantum in children and can cause primary infection or reactivation in immunocompromised patients. Although serologic testing is not the preferred means of establishing a diagnosis of HHV-6 infection, IgG seroconversion, the demonstration of anti-HHV-6 IgM, or a four-fold rise in IgG antibody titers using paired sera may indicate recent infection. Commercial assays do not typically distinguish between variants A and B. Because of the ubiquitous nature of HHV-6, most people have been exposed to the virus by two years of age. Therefore, a single positive result for anti-HHV-6 IgG may not be able to differentiate recent infection from remote exposure.

The most commonly used molecular test for the laboratory diagnosis of HHV-6 is NAAT and at least one multiplex test platform for this is FDA-cleared (Table 55). However, qualitative NAAT does not differentiate replicating from latent virus. HHV-6 DNA quantification may be useful in this regard, as well as in monitoring response to antiviral therapy. HHV-6 may be shed intermittently by healthy and immunocompromised hosts. Therefore, detection of HHV-6 in blood, body fluids or even tissue does not definitively establish a diagnosis of disease caused by HHV-6. Chromosomally integrated HHV-6 (ci-HHV-6), which results in high HHV-6 levels in virtually all clinical specimens, may lead to an erroneous diagnosis of active infection.

Although no longer routinely performed, HHV-6 can be cultured from peripheral blood mononuclear cells (and other clinical specimens) [453]. However, viral isolation is labor-intensive, taking up to 21 days. The detection time for HHV-6 culture can be shortened to 1 to 3 days with the use of shell vial culture assay. In addition to a long processing time, culture-based assays suffer from poor sensitivity and do not differentiate between variants A and B, which is not useful clinically. If tissue biopsy is performed, HHV-6 antigens can be targeted by immunohistochemical or *in situ* hybridization tests in formalin-fixed, paraffin-embedded tissues.

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Table 55. Laboratory Diagnosis of Human Herpes Virus-6 (HHV-6) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Cerebrospinal fluid	Sterile, preservative-free container, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Saliva	Sterile, preservative-free container, RT, ≤24 h
	Whole blood, peripheral blood mononuclear cells	EDTA or citrate tube, RT, ≤2 h

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Herpes simplex virus

Herpes simplex virus types 1 (HSV-1) and -2 (HSV-2) are common causes of dermal and genital lesions but may also result in central nervous system disease or congenital infections. Serology should not be used as a primary diagnostic test but may assist in determining a patient's exposure status to HSV-1/-2. The presence of IgG-class antibodies to the HSV-1/-2 glycoprotein G antigen indicates previous exposure to the corresponding serotype of the virus. Positive IgG results do not differentiate past from current, active infection unless seroconversion is determined by testing acute and convalescent phase specimens. A fourfold increase in anti-HSV IgG levels may suggest recent exposure; however, most commercial assays no longer yield a titered result that can be used quantitatively. The presence of IgM-class antibodies to HSV suggests primary infection, however, anti-HSV IgM reactivity is often absent at the time of lesion development, with IgM seroconversion occurring 1 to 2 weeks after infection. Also, commercial IgM assays are not able to reliably distinguish between infection with HSV-1 and HSV-2 and may be falsely positive due to other viral infections, alloantibodies present during pregnancy, or autoimmune disorders.

NAAT is the most sensitive, specific and rapid test for diagnosis of HSV-associated skin or mucosal lesions and can detect and distinguish HSV-1/-2 (Table 56; refer to genital infections section). For collection of specimens, a viral culture transport swab should be vigorously rubbed over the base of the suspect skin or mucosal lesion; the vesicle should be unroofed to expose the base. Older, dried and scabbed lesions are less likely to yield positive results. Culture and direct fluorescent antibody testing are less sensitive than NAATs, especially for the detection of HSV-1/-2 from CSF.

HSV NAATs are now considered the gold-standard to diagnose HSV central nervous system disease and an FDA-cleared option is available (Simplexa® HSV1-2 Direct, DiaSorin Molecular, Inc.) [454]. Testing should detect and distinguish HSV-1/-2, as type 1 is most commonly associated with encephalitis and type 2 with meningitis. Viral culture of CSF is insensitive for

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diagnosis of HSV central nervous system disease and should not be used to rule-out HSV encephalitis/meningitis.

Table 56. Laboratory Diagnosis of Herpes Simplex Virus (HSV) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT	Scraping of base of dermal or mucosal lesion collected using a swab	Place into viral transport medium[1], RT, ≤24 h
	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
Serology[2]	Serum	Clot or SST tube, RT, ≤2 h
Direct fluorescent antibody test	Vesicle fluid on slide	Place in sterile, preservative-free container, RT, ≤24 h
Culture	Scraping of base of dermal or mucosal lesion collected using a swab (CSF should not be cultured due to poor sensitivity)	Place into viral transport medium[2], RT, or on wet ice, ≤24 h

[1] M4 or M5 media acceptable; do not use calcium alginate-tipped swab, wooden shaft swab, or transport swab containing gel due to inhibition of PCR.

[2] Evaluation for HSV-specific IgM antibody is not recommended as a means to establish recent or acute infection; NAAT or culture is preferred.

RT, room temperature; SST, serum separator tube.

Varicella-zoster virus

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

Although viral culture can be used to recover VZV from clinical specimens, it may take up to 14 days for cytopathic effect (CPE) to be observed. Due to this delay in turnaround time, NAATs have become routinely used for the diagnosis of VZV and offer the most sensitive and rapid approach to detect the virus (Table 57). For dermal lesions that are suspected to be associated with VZV infection, a culture transport swab should be vigorously rubbed on the base of the suspect skin lesion; the vesicle should be unroofed to expose the base. The swab should then be placed in viral transport media and transported to the testing laboratory. A less sensitive method for diagnosis is detection of viral antigens by direct fluorescent antibody stain of lesion scrapings.

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Suspected VZV-associated skin lesions should be clinically differentiated from smallpox. Information regarding clinical manifestations of smallpox, including differentiation from VZV pocks, and laboratory testing can be found on the CDC website (https://www.cdc.gov/smallpox/index.html).

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

Table 57. Laboratory Diagnosis of Varicella-Zoster Virus (VZV) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT	Scraping of base of dermal lesion collected using swab	Viral transport medium[1], RT, ≤24 h
	Cerebrospinal fluid	Sterile, preservative tube, RT, ≤2 h
Serology[2]	Serum	Clot or SST tube, RT, ≤2 h
Direct fluorescent antibody test	Vesicle fluid on slide	Place in sterile container, RT, ≤24 h

[1] M4 or M5 media acceptable; do not use calcium alginate-tipped swab, swab with wood shaft, or transport swab containing gel due to inhibition of PCR.
[2] Evaluation for VZV-specific IgM antibodies is not recommended as a means to establish recent or acute infection; NAAT or culture is preferred.
RT, room temperature; SST, serum separator tube.

Hepatitis viruses

Hepatitis A and E Viruses

Diagnosis of acute hepatitis A virus (HAV) infection is confirmed by detecting HAV IgM antibody, which may persist for up to 6 months after exposure (Table 58). However, false-positive HAV IgM antibody results can occur due to low positive predictive value of such immunoassays used in populations with low prevalence (eg, <5%) of acute hepatitis A [455], and NAAT for HAV RNA may be necessary to confirm diagnosis. The presence of HAV IgG antibody indicates either past or resolved hepatitis A infection or immunity to this viral infection from vaccination. Alternatively, the same hepatitis A status can be deduced by the presence of HAV total antibodies in an asymptomatic patient with normal liver tests and/or absence of HAV IgM antibody [456].

Hepatitis E is usually a foodborne illness in developing countries due to ingestion of hepatitis E virus (HEV) transmitted in contaminated food and water. However, such infection in developed countries may be encountered in return travelers (acute hepatitis E) or organ transplant recipients (acute or chronic) [457]. Since presentation of acute hepatitis A and E are indistinguishable clinically from one another, diagnosis of the latter is made usually by presence of HEV IgM antibody (appearing by 4 to 6 weeks after exposure and lasting for 2 to 4 months) and absence of HAV IgM antibody in serum or plasma. HEV IgG antibody is detectable in serum and plasma

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usually by 4 weeks after clinical presentation. However, with delayed humoral response in organ transplant recipients who are immunosuppressed from anti-rejection therapy and suspected to have acute hepatitis E, diagnosis may need to be made with molecular assays for detection of HEV RNA in serum or plasma. Individuals with ≥3 months of HEV viremia are considered to have chronic hepatitis E, and quantification of HEV RNA in serum or plasma can be used to monitor disease progression and response to antiviral therapy. Currently, only laboratory-developed immunoassays and NAAT are available in the U.S. for the diagnosis or monitoring of hepatitis E.

Hepatitis B, C, and D Viruses

Hepatitis B surface antigen (HBsAg) may be detected in the presence of acute or chronic hepatitis B virus infection [458]; it indicates that the person is infectious. In acute infection, its appearance predates clinical symptoms by 4 weeks and it remains detectable for one to six weeks. The tests for detecting hepatitis B and D disease are primarily serologic and molecular (Table 59). Clinicians should check the clinical laboratory test catalog or consult with the performing laboratory on the minimum volumes of blood needed, as some molecular assays require more blood than others for testing.

The presence of hepatitis B surface (HBs) antibody 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, HBsAg and HBs antibody are not detectable simultaneously in serum or plasma.

Hepatitis B core (HBc) IgM antibody appears during acute or recent hepatitis B virus infection and remain detectable for about six months. A serologic "window" occurs from 6 to 8 months after infection when HBsAg disappears and HBs antibody becomes undetectable. During this "window" period, infection can be diagnosed by detecting HBc IgM antibody.

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

A chronic hepatitis B virus carrier state is defined by persistence of HBsAg for at least 6 months. In patients with chronic hepatitis B, the presence of hepatitis B e antigen (HBeAg) in serum or plasma is a marker of high viral replication levels in the liver. Loss of HBeAg and emergence of antibody to hepatitis B e antigen (i.e., HBe antibody) is usually associated with improvement of underlying hepatitis and a reduction in the risk of hepatocellular carcinoma and cirrhosis. Alternatively, disappearance of HBeAg may denote the emergence of a precore mutant virus; high concentrations of HBsAg and HBV DNA, in the absence of hepatitis B e antigen and presence of HBe antibody suggest the presence of a HBV precore mutant virus. Hepatitis B viral DNA is present in serum or plasma in acute and chronic hepatitis B infection [459]. Quantification of

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HBV DNA in serum or plasma may be included in the initial evaluation and management of chronic hepatitis B infection, especially when the serologic test results are inconclusive or when deciding treatment initiation and monitoring patient's response to therapy. Other molecular laboratory tests used in the diagnosis and management of chronic hepatitis B infection have been reviewed in the published literature and include assays for determining viral genotype, detection of genotypic drug resistance mutations, and core promoter/precore mutations [459].

Detection of HBs antibody in the absence of HBc total antibodies distinguishes vaccine-derived immunity from immunity acquired by natural infection (in which both HBs antibody and HBc total antibodies are present). Current commercially available assays for detecting HBs antibody yield positive results (qualitative) when antibody levels are ≥10 mIU/mL (or ≥10 IU/L) in serum or plasma, indicating post-vaccination immunity (protective antibody level). Quantitative HBs 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 virus antigen (HDV Ag), HDV IgM and total antibodies are detectable in plasma or serum (Table 59). In acute hepatitis B and D co-infection, the same serologic markers (ie, HDV Ag, HDV IgM and total antibodies) are detectable along with HBc IgM antibody. HDV total antibody testing followed by HDV NAAT for antibody-positive specimens is recommended for HBsAg-positive individuals at risk for HDV infection, including those with HIV infection, persons who inject drugs, men who have sex with men, and immigrants from areas of high HDV endemicity [460]. Since HDV is an RNA virus, quantification of HDV RNA in plasma or serum has been used clinically to monitor progression of chronic hepatitis D and response to antiviral therapy [461]. Currently, only laboratory-developed immunoassays and NAAT are available in the U.S. for the diagnosis or monitoring of hepatitis D.

The diagnosis of HCV infection usually begins with an immunoassay for detection of HCV IgG or total antibodies in serum or plasma. Antibody may not be detectable until 6 to 10 weeks after the onset of clinical illness. Individuals with negative HCV antibody screening test results do not need further testing for hepatitis C (Table 60), except in immunocompromised individuals (in whom development of HCV IgG antibody may be delayed for up to 6 months after exposure) or those with suspected acute HCV infection. Those with positive initial HCV IgG or total antibody test results should undergo confirmatory or supplemental testing for HCV RNA with NAAT. Supplemental HCV IgG antibody assays can confirm the presence of HCV antibodies in patients with positive initial HCV IgG or total antibody test results, but none of these assays are FDA–approved currently for clinical use in the U.S. Per current recommendations from the U.S. Centers for Disease Control and Prevention [462], the following individuals should be screened for HCV infection: 1) all adults aged ≥18 years at least at least once in a lifetime, and 2) all pregnant women during each pregnancy, except in settings where prevalence of HCV infection is <0.1%. In addition, regardless of age or setting prevalence, all persons with risk factors should be tested for hepatitis C, with periodic testing while risk factors persist. Any person who requests hepatitis C

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testing should receive it, regardless of disclosure of risk, because many persons might be reluctant to disclose stigmatizing risks.

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 or quantitatively (by reverse-transcription PCR or transcription-mediated amplification methods). Highly sensitive molecular assays for quantification of HCV RNA in serum or plasma (limit of detection of ≤25 IU/mL) are necessary to monitor patient's virologic response and to determine cure (i.e., sustained virologic response) from antiviral therapy. Determination of HCV genotype and subtypes (i.e., 1 to 6 and 1a vs. 1b) is used to guide the choice and duration of antiviral therapy and predict the likelihood of response to therapy, as different genotypes and subtypes varying in virologic response to current treatment regimens and in likelihood of antiviral resistance before or during direct-acting antiviral treatment (DAA therapy). Pre-treatment testing for HCV genome-specific resistance-associated substitutions (RAS) by conventional (Sanger) or next-generation sequencing assay methods is recommended by the U.S. FDA and/or current clinical practice guideline (https://www.hcvguidelines.org/evaluate/resistance) prior to initiating certain DAA therapy combinations for infection due to certain HCV genotypes: 1) HCV NS3 RAS for simeprevir in genotype 1 infection, and 2) HCV NS5A RAS for elbasvir-grazoprevir or ledipasvir/sofosbuvir in genotype 1a infection, and daclatasvir/sofosbuvir or velpatasvir/sofosbuvir in genotype 3 infection. Per current recommendations from U.S. FDA (https://www.fda.gov/Drugs/DrugSafety/ucm522932.htm), all patients prior to initiating DAA therapy should be screened for evidence of prior or current HBV infection (positive for HBc total antibodies and/or HBsAg), so that such affected patients can be monitored and managed appropriately for reactivation of HBV during and after DAA therapy.

A human genomic polymorphism interleukin-28B (IL-28B) genotype CC (within the interferon gamma promoter gene region of human chromosome 9) is associated with good likelihood of spontaneous resolution of HCV infection in acutely infected individuals as well as high probability of sustained viral response in those receiving interferon-based combination therapy for chronic HCV infection. As interferon-based therapy is seldomly used to treat chronic hepatitis C, IL-28B genotype testing of whole blood or buccal cells is used occasionally to predict likelihood of spontaneous resolution of acute HCV infection.

Table 58. Laboratory Diagnosis of Hepatitis A and E

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues
Serology	HAV IgM antibody HAV IgG antibody HAV total antibodies HEV antigen HEV IgM antibody HEV IgG antibody	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h

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NAAT	HAV RNA, quantitative	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h SST tube, RT, ≤2 h
	HEV RNA, quantitative (viral load)		

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Table 59. Laboratory Diagnosis of Hepatitis B and D

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues
Serology	HBs antigen	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h
	HBs antibody		
	HBc total antibodies		
	HBc IgM antibody		
	HBe antigen		
	HBe antibody		
	HDV antigen		
	HDV IgM antibody		
	HDV IgG antibody		
	HDV total antibodies		
NAAT	HBV DNA, quantitative (viral load)	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h SST tube, RT, ≤2 h
	HDV RNA, quantitative (viral load)		

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Table 60. Laboratory Diagnosis of Hepatitis C Virus (HCV)

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues
Serology	HCV IgG antibody	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h
	HCV total antibodies		
	HCV IgG antibody confirmation		
NAAT	HCV RNA, qualitative	Plasma Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h SST tube, RT, ≤2 h
	HCV RNA, quantification (viral load)		
	HCV genotyping		
	HCV NS3 resistance-associated substitutions (genotype 1 and 3 only)		
	HCV NS5a resistance-associated substitutions (genotype 1and 3 only)		
	HCV NS5b resistance-associated substitution (genotype 1 and 3 only)		

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Retroviruses

Human immunodeficiency viruses (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, integrase (*pol* – p66, p51, p31), and envelope

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glycoproteins (*env* – pg160, gp120, gp41). HIV viruses are classified based on the relatedness of their genome into types 1 and 2, groups, and clades. HIV-1 is categorized into groups M, O, and P, with M being most common [463, 464]. HIV-1 is more common than HIV-2 in the U.S., but the latter should be considered in persons who were born in, have traveled to, have received blood products from, or have had a sexual partner from West Africa, as well as those who have been similarly exposed to HIV-2-infected persons in any geographic area.

After exposure to HIV, HIV RNA is detectable in plasma by 10 to 12 days, followed by appearance of HIV p24 antigen in serum or plasma at 15 to 17 days. Depending on the sensitivity of the serologic assays used, HIV-specific antibodies are detectable in serum or plasma at the earliest at 21 days after exposure. Performing a HIV RNA test after a negative initial antibody and/or antigen test in persons suspected of acute infection may therefore be helpful. Due to the time course of test positivity and the possibility of seronegativity, laboratory diagnosis of primary (acute) HIV infection is usually based on qualitative detection of HIV RNA or proviral DNA or a high quantitative HIV RNA (viral load) result (typically $>10^5$ copies/mL) (Table 61) [465]. However, in the setting of non-acute HIV infection, HIV viral load assays should be used with caution for diagnosis of HIV infection because of the possibility of false-positive results. Since false-positive results are generally of low copy number (<200 copies/mL), low copy number results should prompt retesting of a second specimen. Notably, because there is a 10- to 12-day period after infection when serologic markers are not detectable, testing another specimen 2 to 4 weeks later should be considered if initial antibody, antigen, or RNA tests are negative. NAAT is not 100% sensitive in individuals with established HIV infection due to viral suppression, either naturally or therapeutically, or improper specimen collection/handling. If NAAT is used to make a diagnosis of acute HIV infection, subsequent HIV-1 seroconversion by conventional serologic testing is recommended.

In the neonate, serologic testing is unreliable due to persistence of maternal antibodies; quantitative HIV RNA testing is as sensitive as qualitative HIV RNA or proviral DNA testing for the diagnosis of HIV infection [466]. NAAT is recommended at 14 to 21 days, 1 to 2 months, and 4 to 6 months after birth, in infants born to HIV-1-infected mothers who received antiretroviral therapy (ART) during pregnancy and had sustained undetectable HIV-1 RNA in plasma. However, high-risk neonates (i.e. no prenatal care, no antepartum or intrapartum ART, ART initiated late in pregnancy, history of acute HIV during pregnancy, or detectable HIV-1 RNA in infected mothers near time of delivery) should have additional NAAT at birth and 8 to 10 weeks (i.e. 2-4 weeks after completing neonatal ART). Since the availability of HIV serologic assays in the 1980s, initial HIV tests have evolved to the current antigen-antibody (Ag-Ab) combination and discriminatory immunoassays in which recombinant and synthetic HIV peptide antigens are used to detect HIV p24 antigen and HIV-specific IgM and IgG antibodies. Such assays generally yield positive results by 4 to 6 days after positive NAAT results. HIV Ag-Ab discriminatory immunoassays (eg, BioPlex 2200 HIV Ag-Ab; Bio-Rad Laboratories, Inc.) have the advantage over Ag-Ab

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combination immunoassays in their ability to discriminate among HIV-1 p24 antigen, HIV-1 antibodies, and HIV-2 antibodies.

HIV-1 p24 antigen is detected in serum or plasma usually by 14 to 16 days after infection (before antibody becomes detectable), and it typically decreases below detection limit thereafter, limiting the utility of p24 antigen testing alone for the diagnosis of HIV infection. The U.S. Association of Public Health Laboratories and the Centers for Disease Control and Prevention now recommend the use of HIV Ag-Ab combination immunoassays for initial testing of individuals for diagnosis of HIV infection in an algorithmic testing approach [463, 464]. The testing algorithm using such assays recommends serum or plasma specimens with reactive initial test results to be tested in reflex with HIV antibody differentiation immunoassays that can distinguish between HIV-1 and HIV-2 antibodies. If the antibody differentiation assay result is negative, further testing with a qualitative or quantitative NAAT is recommended to rule out acute HIV infection. If the differentiation assay is positive, viral load testing (as wells as CD4 cell count determination) is recommended to guide management. Alternatively, if a Ag-Ab discriminatory immunoassay is used as the initial test and only the HIV-1 p24 antigen result is reactive, then such specimens can be tested subsequently with NAAT, whereas those specimens that are reactive for HIV-1 or HIV-2 antibodies can be tested subsequently with HIV antibody differentiation assays. Use of HIV antibody-only immunoassays is limited to the diagnosis of non-acute HIV-1 infection (Table 61), since these assays can detect only HIV-1 and HIV-2 antibodies. Serum or plasma specimens that are reactive with such initial serologic assays can be tested further with HIV antibody differentiation assays for confirmation. Individuals with initially reactive results from whole blood, serum, plasma, or saliva specimens tested with rapid HIV antibody-only or Ag-Ab combination assays (eg, point-of-care rapid tests) should be tested further with laboratory-based Ag-Ab immunoassays to determine the HIV infection status as described above. The current APHL / CDC HIV testing algorithm no longer recommends supplemental HIV-1 antibody testing by Western blot because of the subjectivity, labor intensity, and limited access of these manual assays.

Serum or plasma specimens that are reactive by initial HIV Ag-Ab immunoassays but negative or indeterminate by supplemental antibody differentiation immunoassays should be tested subsequently with FDA-approved qualitative HIV-1 or HIV-1/-2 RNA NAAT for detection of possible early or acute HIV infection. Some clinical laboratories have adapted commercially available quantitative HIV-1 NAAT for qualitative detection of HIV-1 RNA in plasma specimens. Quantification of HIV RNA in plasma (viral load) is the current standard approach to guide initiation of antiretroviral therapy as well as monitoring antiviral response and disease progression in infected individuals. Currently, only laboratory-developed quantitative HIV-2 NAAT are available for the monitoring of HIV-2 infection in the U.S.

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

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therapy. Genotypic resistance testing is recommended generally for treatment-naïve patients, while phenotypic resistance testing is reserved mainly for treatment-experience patients whose genotypic HIV resistance profiles show multiple resistance-associated mutations that could not predict an effective antiviral drug combination.

Table 61. Laboratory Diagnosis of HIV-1/-2 Infection

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues
Serology (rapid, point-of-care tests)	HIV-1 and HIV-2 antibodies	Oral fluid (saliva) Whole blood (fingerstick or venipuncture)	Not applicable
	HIV-1 and HIV-2 Ag-Ab combination	Whole blood (fingerstick or venipuncture)	Not applicable
Serology (laboratory-based tests)	HIV-1 and HIV-2 antibodies only HIV-1 and HIV-2 Ag-Ab combination HIV-1 and HIV-2 antibody differentiation	Plasma[1] Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h
NAAT	HIV-1 DNA and RNA, qualitative HIV-2 DNA and RNA, qualitative	Whole blood Plasma[2]	EDTA or citrate tube, RT, ≤2 h EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	HIV-1 RNA, qualitative HIV-1/-2 RNA, qualitative	Plasma[1] Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h
	HIV-1 RNA, quantitative (viral load) HIV-2 RNA, quantitative (viral load) HIV-1 genotypic or phenotypic drug resistance	Plasma[1]	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h

[1] For viral load testing, blood collected in PPT tube should be processed within 6 h of collection to separate plasma from cells prior to transport. Since PCR does not differentiate between such proviral DNA and cell-free viral RNA, leakage of proviral DNA from cells during storage in PPT tube may cause falsely elevated plasma HIV RNA level results.
[2] NAAT are commercially available to detect non-integrated HIV proviral DNA present in cell-free plasma.
PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube

Human t-lymphotropic viruses (htlv)

HTLV is a group of reverse-transcribing RNA viruses within the family of retroviruses. Among the 4 known species in this group of viruses (HTLV-1 through HTLV-4), only HTLV-1 has been shown definitively to be associated with human diseases as it causes human adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy (HAM, also known as tropical spastic

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paraparesis, TSP). Diagnosis of HAM/TSP relies on a combination of clinical features, laboratory confirmation of HTLV-1 infection, and exclusion of other neurologic disorders presenting with spastic paraparesis. Various serologic screening assays using purified viral lysates and/or recombinant synthetic peptides are commercially available to detect HTLV-1 and HTLV-2 antibodies present in the plasma and serum (Table 62). Due to the potential for false positive results in such initial tests, supplemental serologic assays are used to confirm the initial reactive results and to differentiate between HTLV-1 and HTLV-2 antibodies. Current World Health Organization diagnostic guidelines for HAM/TSP include laboratory detection of HTLV-1 antibodies or antigens in both blood and CSF as a criterion for diagnosis [467].

HTLV-1 RNA is present at very low levels (<100 copies/mL) in plasma of infected individuals, regardless of stage of infection, precluding it as a useful marker for disease progression [468]. In contrast, HTLV-1 proviral DNA can be detected and quantified in peripheral blood mononuclear cells (PBMC) of symptomatic and asymptomatic infected individuals [469, 470]. Although individuals with high proviral loads (e.g., >50,000 copies/10^6 PBMC) have been shown to have a higher risk of disease progression [471], there is no current consensus on a proviral load threshold to predict disease outcome or guide patient management, mainly due to variation associated with the available quantification assays (e.g., viral genomic target, assay method, quantification range) used by different investigators and laboratories and the lack of an international reference standard for HTLV-1 proviral DNA. At present, non-standardized qualitative HTLV-1 proviral DNA tests are available as laboratory-developed assays performed on whole blood specimens at various commercial reference testing laboratories or research laboratories.

Table 62. Laboratory Diagnosis of HTLV-1 Infection

Diagnostic Procedures	Viral Marker	Optimal Specimens	Transport Issues
Serology	HTLV-1/-2 antibody	Cerebrospinal fluid Plasma Serum	Sterile, preservative-free tube, RT, ≤24 h EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h Clot or SST tube, RT, ≤2 h
	HTLV-1/-2 antibody confirmation and differentiation		
NAAT	HTLV-1 proviral DNA, qualitative	Whole blood	EDTA or citrate tube, RT, ≤2 h
	HTLV-1 proviral DNA, quantitative		

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube

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Polyomaviruses

Bk virus

BK virus is a polyomavirus that may cause allograft nephropathy in renal transplant recipients and hemorrhagic cystitis, especially in bone marrow transplant patients. A definitive diagnosis of these conditions requires renal allograft biopsy with *in situ* hybridization for BK virus.

Detection of BK virus by NAAT in plasma may provide an early indication of allograft nephropathy, and there is currently one FDA-cleared NAAT for BKV quantification in plasma (Cobas BKV, Roche Molecular Systems, Inc.) (Table 63) [472]. Urine cytology or quantitative NAAT may be used as a screening test, and if positive, may be followed by BK viral load testing of plasma, which has a higher clinical specificity. Each institution must establish a threshold for identifying patients at highest risk of BK virus-associated nephropathy. Urine NAAT for BK virus may be more sensitive than detection of decoy cells (virus-infected cells shed from the tubules or urinary tract epithelium) using urine cytology, as BK virus DNA is typically detectable earlier in the urine than are decoy cells. However, shedding of BK virus in urine is common. Therefore, if used as a screening test, only high levels (i.e., above a laboratory-established threshold that correlates with disease) should be considered significant. Urine testing for BK virus places the laboratory at risk for specimen cross-contamination, as extremely high levels of virus in the urine may lead to carryover between specimens, and potentially, false-positive results.

Table 63. Laboratory Diagnosis of BK Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Cytology	Urine	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, ≤24 h
NAAT, quantitative (viral load)	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum	SST tube, RT, ≤2 h
	Urine	Sterile, preservative-free container, RT, ≤24 h
Histopathology	Tissue	Sterile, preservative-free container, RT, ≤24 h

PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Jc virus

John Cunningham (JC) virus is the etiologic agent of progressive multifocal leukoencephalopathy (PML), which is a fatal, demyelinating disease of the central nervous system that occurs in immunocompromised hosts. Histologic examination of brain biopsy tissue may reveal characteristic pathologic changes; however, *in situ* hybridization for JC virus is often required to

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confirm the diagnosis. Detection of JC virus DNA in CSF specimens by NAAT has largely replaced the need for tissue biopsy for laboratory diagnosis of PML (Table 64). A serologic test (STRATIFY JCV™, Quest Diagnostics, Inc.) is now FDA-cleared for screening patients who are considering treatment with certain immune-modulating therapies (eg. natalizumab). A positive result by this test is indicative of prior exposure to JCV, and potentially elevated risk of developing PML, if initiating treatment with the immune-modulating drug, natalizumab.

Table 64. Laboratory Diagnosis of JC Virus Infection

Diagnostic Procedure	Optimal Specimen	Transport Issues
NAAT	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
Histopathology	Tissue	Sterile, preservative-free container, RT, ≤24 h

RT, room temperature.

Respiratory viruses

Adenovirus

In otherwise healthy individuals, adenoviruses may cause mild, self-limiting respiratory illness or conjunctivitis, with most cases being diagnosed on clinical grounds. Occasionally, adenovirus infections in immunocompetent hosts can result in severe disease, especially in children with asthma. In immunocompromised patients, adenoviruses may cause pneumonia, disseminated infection, gastroenteritis, hemorrhagic cystitis, meningoencephalitis, or hepatitis.

The diagnosis of adenoviral infections Is typically made using NAAT, viral culture and/or histopathology (Table 65). Viral culture has a long turn-around time (~5 to 7 days), but this can be reduced by using shell vial technology. Plasma viral load (assessed by quantitative NAAT) may be useful as a marker for preemptive therapy, to diagnose adenovirus-associated disease, and to monitor response to antiviral therapy in some immunocompromised populations. Adenovirus is included in certain FDA-cleared multiplex respiratory panels.

Table 65. Laboratory Diagnosis of Adenovirus Infection

Diagnostic Procedures	Optimum Specimens	Transport Issues
NAAT	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen, stool, conjunctiva swab	Sterile, preservative-free container or viral transport medium, RT, ≤24 h
	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h

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Antigen detection, rapid	Nasopharyngeal swab, respiratory specimen	Sterile, preservative-free container or viral transport medium, RT, ≤24 h
Antigen detection (Adenovirus types 40 and 41)	Stool	Sterile, preservative-free container, RT, ≤24 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen, stool, cerebrospinal fluid	Sterile, preservative-free container or viral transport medium, RT, ≤24 h

PPT, plasma preparation tube; RT, room temperature

Coronaviruses

Coronaviruses are host-specific and can infect a variety of animals as well as humans. Four distinct sub-groupings have been described, known as alpha, beta, gamma and delta. Among these, there are seven coronaviruses that have been associated with human disease; Human coronaviruses (HCoV)-229E and -NL63 (subgroup alphacoronavirus), HCoV-OC43 and -HKU1 (subgroup betacoronavirus, lineage A), as well as three associated with more severe disease, including severe acute respiratory syndrome CoV-1 (SARS-CoV; subgroup betacoronavirus, lineage B), Middle East respiratory syndrome CoV (MERS-CoV; subgroup betacoronavirus, lineage C), and SARS-CoV-2 (subgroup betacoronavirus, lineage B).

Human coronaviruses 229E, NL63, OC43 and HKU1 are generally associated with mild symptoms of rhinorrhea, congestion, sore throat, sneezing, cough and may present with fever. In children, these HCoVs have also caused exacerbation of asthma and otitis media. Respiratory secretions or nasopharyngeal (NP) swabs placed in viral transport media (VTM) are the specimens of choice for detection of common HCoVs. Diagnostic tests include NAATs, and common HCoVs are often included in multiplex respiratory panels, which may be beneficial in immunocompromised hosts or those with severe illness.

Suspected cases of SARS-CoV and MERS-CoV require immediate notification to the laboratory and public health officials. Guidance for testing can be found at www.cdc.gov/sars/index.html and www.cdc.gov/coronavirus/MERS/index.html. Fortunately, neither SARS-CoV-1 nor MERS-CoV have been associated with widespread transmission or disease. Testing for both viruses is limited to public health laboratories.

SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), resulted in a global pandemic in 2020. SARS-CoV-2 can be recovered in certain eukaryotic cell lines; however, viral culture should not be utilized for routine diagnosis as cultivation of SARS-CoV-2 requires biosafety level 3 practices. In response to the pandemic, hundreds of diagnostic tests, including molecular, antigen, and serology received emergency use authorization from the FDA (Table 66). In general, molecular tests (e.g., real-time PCR) have been considered the standard approach to diagnosing acute infection with SARS-CoV-2 [473]. Following exposure to the virus, the

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incubation period is 3 to 5 days, on average, and molecular tests have demonstrated high sensitivity and specificity for detection of SARS-CoV-2 RNA from respiratory samples, such as nasopharyngeal (NP) swabs, anterior nares swab and mid-turbinate nasal swabs. Saliva samples have also been used to detect SARS-CoV-2 RNA. Molecular testing should only be performed for diagnosis of acute infection, as viral RNA can be detected for weeks or months in certain patients, especially those who are immunocompromised.

Although historically viewed as possessing limited utility in diagnosing respiratory infections, rapid antigen tests have been widely used in the diagnosis of COVID-19. These tests offer low sensitivity in the first few days following infection; however, in symptomatic patients, antigen tests have demonstrated a sensitivity of 70% to 80% [474]. However, in the asymptomatic population, the sensitivity of antigen tests has ranged from 40% to 50% [475]. A positive antigen test during the first week of symptoms is generally diagnostic for COVID-19, but a negative result should be confirmed by a molecular test, especially in the setting of compatible symptoms. Antigen tests have also been studied as potential measures of infectivity and variable results have been observed. In general, antigen tests are likely to be positive when high amounts of the virus are present, but SARS-CoV-2 has been cultured in some cases where antigen testing was negative.

Although SARS-CoV-2 serologic testing plays a minimal role for diagnosis of COVID-19, there continues to be significant interest in using this method as a means to determine protective immunity against re-infection and significant disease. SARS-CoV-2-specific IgM and IgG become detectable approximately 7 to 10 days following symptom onset, with IgM peaking at approximately 4 to 6 weeks prior to becoming undetectable [476]. In contrast, IgG levels peak at 2 to 4 months post-infection, although this peak and the duration of detectable IgG varies dramatically based on multiple factors, including disease severity, patient immunostatus, and the serologic assay characteristics among others. Notably, there are over 80 SARS-CoV-2 serologic assays currently available, which differ in the methods used (i.e., chemiluminescence [CIA], lateral flow [LFA], enzyme-linked immunoassay [ELISA]), antibody class detected (i.e., IgM, IgG, IgM/IgG, total Ig undifferentiated, neutralizing antibodies), targeted SARS-CoV-2 antigen (i.e., nucleocapsid, spike, receptor-binding domain, or a combination), specimen type (i.e., serum, plasma, fingerstick whole blood, dried blood spot) and result output (i.e., qualitative, semi-quantitative, quantitative), which all impact assay performance characteristics and result interpretation [477, 478]. For example, while anti-nucleocapsid antibody detection can be used to determine prior infection status, antibodies to this antigen decline more rapidly as compared to anti-spike-based serologic assays, so a negative result cannot be used to definitively exclude past infection [479]. In contrast, reactivity of anti-spike-based serologic assays cannot be used to differentiate between past infection versus vaccination, given that all SARS-CoV-2 vaccines target spike protein epitopes.

Use of SARS-CoV-2 serologic assays to measure protective immunity remains of significant interest for the purpose of guiding re-vaccination decisions, particularly for individuals who are at high risk of severe COVID-19 (i.e., patients with hematologic malignancy, recent transplant

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recipients, etc.) and who have a poor humoral response to prior vaccination or infection. The challenge, however, is that although anti-spike antibodies are largely accepted as a dependable correlate of protection (CoP; defined as an immunologic marker able to predict vaccine efficacy against a clinical endpoint), there is no universally accepted CoP 'threshold' to discriminate disease risk, unlike for other vaccine preventable diseases [480]. Much of the SARS-CoV-2 CoP data has emerged from vaccine clinical trials, including a recent study from the Coronavirus Efficacy or COVE Trial, which serially measured binding and neutralizing antibodies in patients fully vaccinated with mRNA-1273 [481]. This study showed that higher anti-spike antibody levels are associated with lower risk of breakthrough infection, which suggests that the SARS-CoV-2 CoP will be more of a 'continuum model,' rather than being based on a single threshold cut-off scenario. Currently however, due to the lack of defined CoP thresholds and the absence of standardization across SARS-CoV-2 serologic assays, the FDA, CDC, and other societies recommend against use of serologic assays to measure 'immunity' or use of these results to guide vaccination decisions.

Table 66. Laboratory Diagnosis of COVID-19

Diagnostic Procedures[1]	Optimum Specimens	Transport Issues
NAAT	Nasopharyngeal swab, mid-turbinate nasal swab, anterior nares swab; lower respiratory specimen (e.g., BAL fluid) validated in certain laboratories	Sterile, preservative-free container or viral transport medium, RT, ≤24 h
Antigen detection, rapid	Anterior nares or mid-turbinate nasal swab	Sterile, preservative-free container or viral transport medium, RT, ≤24 h
Serology	Serum	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Plasma	Clot or SST tube, RT, ≤2 h
	DBS	RT ≤28 days

BAL, bronchoalveolar lavage; DBS, dried blood spots, RT, room temperature.

[1] Culture should not be ordered for diagnosis of COVID-19 as propagation of SARS-CoV-2 outside of a biosafety level 3 (BSL-3) facility poses a safety risk to laboratory personnel.

Influenza

Rapid diagnosis of influenza virus infection (≤48 h following the onset of symptoms) is needed to facilitate early administration of antiviral therapy. The virus may be rapidly detected by NAAT or direct antigen detection from a nasopharyngeal or anterior nares swab (Table 67). Rapid antigen tests suffer from poor sensitivity (50% to 75%) and may perform poorly in detecting certain strains of influenza (especially pandemic H1N1 and swine-associated H3N2 strains) and negative tests should be confirmed by NAAT or culture prior to ruling out influenza infection. During seasons of low prevalence of influenza, false-positive rapid antigen test results are more likely to occur. The performance of influenza assays, including NAAT and rapid antigen tests, varies depending

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on the assay and the circulating strains. Due to good diagnostic sensitivity, NAAT is considered the gold standard for detection of influenza virus in clinical samples. Several FDA-cleared NAAT platforms exist, including multiplex respiratory panels that can detect and differentiate influenza A and influenza B.

Influenza virus can be recovered in routine viral cell culture, but confirmation is needed, typically through the use of hemadsorption and/or hemagglutination techniques. Serologic testing is not useful for the routine diagnosis of influenza due to high rates of vaccination and/or prior exposure.

Table 67. Laboratory Diagnosis of Influenza A and B Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT[1]	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT, ≤24 h
Antigen detection (rapid)	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT, ≤24 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile container or viral transport medium, RT or ideally on wet ice, ≤24 h,

[1] FDA-cleared commercial products are available for rapid NAAT testing for respiratory viruses
RT, room temperature.

Parainfluenza

Parainfluenza viruses are a major cause of croup (laryngotracheobronchitis), bronchiolitis and pneumonia as well as upper respiratory tract infections. Of the 4 antigenically distinct types, types 1 and 2 are most commonly associated with croup syndrome, while type 3 is associated with bronchiolitis and pneumonia. Parainfluenza virus infections account for up to 11% of all hospitalizations in children less than 5 years old [482].

Respiratory secretions or nasopharyngeal (NP) swabs placed in appropriate viral transport media (VTM) are the specimens of choice. Diagnostic tests include culture, which may take 4 to 7 days for recovery of the virus, and NAATs, with parainfluenza now being a common component of commercial respiratory panels.

Human Metapneumovirus (hmpv)

Human metapneumovirus (hMPV) has been shown to cause acute respiratory tract disease in people of all ages. The virus has been associated with cases of bronchiolitis in infants as well as pneumonia, exacerbations of asthma, croup, and upper respiratory infections with concomitant otitis media in children. Most commonly, children present with mild to moderate symptoms. Infection with hMPV is also associated with exacerbations of chronic obstructive pulmonary disease (COPD) pneumonia in adults. When diagnostic tests are required, the specimens of choice

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are respiratory secretions or NP swabs placed in VTM. Diagnostic tests include immunofluorescence assays and NAATs, which are now available in several commercial respiratory panels.

Respiratory syncytial virus

Respiratory syncytial virus (RSV) causes bronchiolitis and/or pneumonia and is most common in infants and young children, although it can cause respiratory illness in adults and severe disease in immunocompromised hosts. NAAT testing has become the diagnostic method of choice, and the preferred specimen types include a nasopharyngeal swab or BAL fluid, if the patient has evidence of lower respiratory tract infection (Table 68). Several FDA-cleared NAAT platforms exist. Although RSV can be recovered in routine viral culture, this approach is time-consuming, and CPE may not be observed for up to 2 weeks. Rapid antigen tests are not generally recommended due to poor sensitivity compared to NAAT.

Serology is not recommended as a diagnostic method in patients with suspected RSV infection. The seroprevalence to RSV is high, and the presence of IgG-class antibodies generally indicates past exposure and immunity.

Table 68. Laboratory Diagnosis of Respiratory Syncytial Virus (RSV) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
NAAT[1]	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile, preservative-free container or viral transport medium, RT, ≤24 h
Culture	Nasopharyngeal aspirate/washing, throat or nasopharyngeal swab, lower respiratory specimen	Sterile, preservative-free container or viral transport medium, RT or ideally on wet ice, ≤24 h,

[1] Commercial products are available for rapid PCR testing for respiratory viruses
RT, room temperature

Vaccine preventable viruses

Measles (rubeola) virus

Although endemic measles was proclaimed eliminated in the United States in 2000 as a result of high vaccination rates and vaccine efficacy (~97% following two doses), travel-associated cases (and spread among unvaccinated individuals) continue to occur (www.cdc.gov/measles/vaccination.html). Immunity to measles is indicated by the presence of IgG-class antibodies to the virus. While diagnosis of recent (acute) measles infection can be made on clinical grounds, supportive laboratory findings include a positive anti-measles IgM result. IgM antibodies are often positive by the time the rash appears, but up to 20% of patients may be serologically negative within the 72 hours post rash onset. Therefore, in suspected measles cases,

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initially seronegative cases during the acute stage, a second specimen collected 72 hours after rash onset should be collected and tested for anti-measles IgM to document seroconversion. IgM antibodies to measles may be detectable for a month or longer following disease onset and may also be positive in recently vaccinated individuals. A serologic diagnosis of acute measles may be established by demonstrating seroconversion of anti-measles IgG antibodies or a four-fold rise in IgG titers between acute (collected at the time of rash onset) and convalescent (collected 10 to 30 days later) specimens (Table 69). Notably however, quantitative or semi-quantitative testing for anti-measles antibodies (i.e., determining a titer) is no longer routinely available in local or reference laboratories. Measles virus can be isolated by culture or detected by NAAT from throat, nasal or nasopharyngeal swabs or urine collected soon after rash onset; such testing is typically limited to public health laboratories [483].

Infrequently, measles infection may lead to development of subacute sclerosing panencephalitis (SSPE) later in life. Measurement of antibodies to measles in CSF is recommended in suspected cases of SSPE. Importantly, efforts should be made to ensure that detected anti-measles antibodies in CSF are due to intrathecal antibody synthesis (e.g., by comparing semi-quantitative antibody levels in CSF and serum or establishing a CSF:serum antibody ratio), rather than due to passive diffusion across the blood brain barrier or blood contamination during a traumatic lumbar puncture [484].

Table 69. Laboratory Diagnosis of Measles (Rubeola) Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology	Cerebrospinal fluid[1]	Sterile, preservative-free tube, RT, ≤24 h
	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Oropharyngeal swab, oral fluid	Sterile, preservative-free container, RT, ≤24 h
	Urine	Sterile, preservative-free container, RT, ≤2r h
	Whole blood	EDTA or citrate tube, RT, ≤2 h
Culture	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Oropharyngeal or nasopharyngeal swab[2], nasal aspirate	Viral transport media, RT or on wet ice, ≤24 h
	Urine	Sterile, preservative-free container, RT, ≤24 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

[1] Measles-specific antibody levels in CSF should be compared to levels in serum to assess for true intrathecal antibody synthesis (ie, via semi-quantitative titer comparison or establishment of a CSF:serum antibody ratio).

[2] Place the swab in viral transport medium, cell culture medium or other sterile isotonic solution (e.g., saline).

SST, serum separator tube; RT, room temperature.

Mumps

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Similar to measles, mumps is considered eliminated in the United States, though travel associated cases among unvaccinated individuals continue to occur, and while effective, the mumps vaccine has a protective rate of ~88% following administration of the 2 doses (www.cdc.gov/mumps/vaccination.html). Immunity to mumps is suggested by the presence of anti-mumps IgG-class antibodies. While mumps infection presents with classic symptoms (e.g., parotitis), diagnosis of infection can be supported by a positive serologic test for anti-mumps IgM antibodies and/or seroconversion or a four-fold rise of mumps IgG antibody levels between acute and convalescent phase sera (Table 70). Ideally, acute phase sera should be collected immediately upon suspicion of mumps virus infection and/or symptom onset and convalescent sera collected approximately 5 to 10 days thereafter. IgM antibodies to mumps typically become detectable during the first few days of illness, peak approximately one week post onset and may remain detectable for a few months. As with serologic testing for measles, quantitative or semi-quantitative (i.e., determining a titer) testing for mumps IgG-class antibodies is no longer routinely available in local or reference laboratories.

Notably, previously immunized patients who are subsequently infected with mumps may not develop a detectable IgM response to the virus. For such individuals, confirmation of mumps infection requires isolation of the virus itself or detection of viral RNA; these tests are largely limited to public health laboratories and the CDC. The preferred specimen source for culture and/or NAAT is an oral or buccal swab around the affected parotid gland and Stensen's duct [485]. Mumps virus RNA may be detected prior to onset of parotitis until 5 to 9 days post symptom onset. Unlike for measles, urine samples are not considered as sensitive for mumps culture or NAAT, as the virus is often not detected in this specimen source until at least 4 days following symptom onset.

Table 70. Laboratory Diagnosis of Mumps Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Oropharyngeal or nasopharyngeal swab[1]	Viral transport medium, RT, ≤24 h
	Parotid (Stensen's) duct/buccal swab[2]	
	Urine	Sterile, preservative-free container, RT, ≤24 h
Culture	Cerebrospinal fluid	Sterile, preservative-free tube, RT but best on wet ice, ≤24 h,
	Oropharyngeal or nasopharyngeal swab[1]	Viral transport medium, RT, ≤24 h
	Parotid (Stensen's) duct/buccal swab[2]	Sterile, preservative-free container, RT, but best on wet ice, ≤24 h

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	Urine	

[1] Place swab in viral transport medium, cell culture medium or other sterile isotonic solution (eg, saline).
[2] Massage parotid gland for 30 seconds and then swab parotid (Stensen's) duct using a viral culture transport swab.
[3] Specimen is associated with lower sensitivity for culture and NAAT.
SST, serum separator tube; RT, room temperature.

Rubella

Rubella (German measles or three-day measles) was officially proclaimed eliminated from the United States in 2004, largely due to intense vaccination efforts. With fewer than 10 cases reported per year, these are often travel associated and sporadic. Serologic testing for detection of anti-rubella antibodies can be used to establish immunity or to provide laboratory-based evidence for rubella infection (Table 71). The presence of IgG antibodies to rubella virus in an asymptomatic individual indicates life-long immunity to infection. Acute rubella infection can be serologically confirmed by documenting seroconversion to IgM and/or IgG positivity or a four-fold rise in anti-rubella IgG titers between acute and convalescent serum specimens. As with measles and mumps serologic assays however, assays providing quantitative titers for antibodies to rubella are not commonly offered at local or reference laboratories.

Only approximately 50% of patients are positive for IgM antibodies to rubella at the time of rash onset, which emphasizes the importance of collecting a convalescent sample. Acute phase serum should be collected upon patient presentation and again 14 to 21 days (minimum of 7) days later. Due to the rarity of rubella in the United States and thus the low pre-test probability of infection, serologic evaluation should only be performed in patients with appropriate exposure risks and a clinical presentation highly suggestive of acute rubella; in patients not meeting these criteria, positive rubella IgM results should be interpreted with caution as they may be falsely positive.

Congenital rubella syndrome (CRS) can be diagnosed by the presence of IgM-class antibodies to rubella in a neonate, alongside symptoms consistent with CRS, appropriate exposure history of the mother, and lack of maternal protective immunity. NAAT for detection of rubella RNA can be performed on throat or nasal swabs and urine, though such testing is largely limited to public health laboratories and/or the CDC. Specimens for NAAT should be collected within seven days of presentation to enhance sensitivity.

Table 71. Laboratory Diagnosis of Rubella

Diagnostic Procedure	Optimal Specimen	Transport Issues
Serology	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Oropharyngeal or nasopharyngeal swabs	Viral transport medium, RT, ≤24 h
	Urine	Sterile, preservative-free container, RT, ≤24 h

SST, serum separator tube; RT, room temperature

Zoonotic viral infections

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Arboviruses

Dengue virus

Dengue virus (DENV) is a flavivirus transmitted by *Aedes* species mosquitos and is most often associated with a febrile illness in travelers returning from endemic regions (e.g., Caribbean, South and Central America, Asia, etc.). Diagnosis of DENV infection is most often established by serologic methods for detection of IgM- and/or IgG-class antibodies to the virus or detection of the DENV non-structural protein 1 (NS1) antigen (Table 72). In cases of primary infection, IgM-class antibodies to DENV are detectable as early as 3 to 5 days post symptom onset and remain detectable for two to three months, whereas IgG antibodies to the virus appear 10 to 12 days after onset and are detectable for months to years [486]. Notably, in secondary or repeat DENV infection, IgM antibodies may not be detectable. An initially negative serologic profile for DENV in a patient for whom dengue fever is strongly suspected should be followed up with repeat serologic evaluation on a serum specimen collected 7 to 10 days after disease onset. Seroconversion to either anti-DENV IgM and/or IgG seropositivity is strongly suggestive of recent infection. However, due to the similar antigenic profiles between members of the *Flavivirus* genus, false positive results for antibodies to DENV may occur in patients with a prior flavivirus infection (e.g., West Nile virus, St. Louis encephalitis virus or Zika virus). Plaque reduction neutralization tests (PRNT) are considered the reference standard for detection of antibodies to arthropod-borne viruses (arboviruses) and provide improved specificity over commercial serologic assays; however, due to the complexity of testing, PRNT is currently only available at select public health laboratories and the CDC.

Following infection with DENV, patients may be viremic for four to six days post symptom onset. Though viral isolation is possible during this timeframe, it is not routinely performed in clinical laboratories [487]. Detection of DENV RNA by NAAT is preferred for acutely ill patients presenting within 7 days of symptom onset (https://www.cdc.gov/dengue/healthcare-providers/testing/testing-guidance.html). Recently, detection of the DENV NS1 antigen, which is secreted from infected host cells as early as 1 day post symptom onset and up to 10 days thereafter, has become an acceptable alternative to NAAT for diagnosis of acute DENV infection.

Table 72. Laboratory Diagnosis of Dengue Virus Infection

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology	Serum	Clot or SST tube, RT, ≤2 h
NS1 Antigen	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum	SST tube, RT, ≤2 h

PPT, plasma preparation tube; SST, serum separator tube.

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West Nile Virus and Other Endemic Neuroinvasive Arboviruses in North America

West Nile virus (WNV), alongside other endemic mosquito and tickborne arboviruses including St. Louis encephalitis, Lacrosse encephalitis, California encephalitis viruses, Powassan virus, among others, can cause systemic and less frequently, severe CNS infections. Laboratory diagnosis of these arboviruses is typically accomplished by detecting virus-specific IgM- and/or IgG-class antibodies in serum and/or cerebrospinal fluid [488] (Table 73). IgM antibodies to these viruses are detectable three to eight days post symptom onset and often taper off 2 to 3 months later, although seropersistence of anti-WNV IgM in serum for up to 12 months has been documented. Seroconversion to anti-WNV IgM and/or IgG positivity between acute and convalescent sera (collected 7 to 10 days apart) is strongly suggestive of a recent infection, whereas the presence of antiviral IgG alone at the time of presentation is indicative of infection at some time in the past and evaluation for an alternative etiology is recommended. Serologic diagnosis of arboviral CNS infections may be established by detection of IgM antibodies in CSF as this class of antibodies do not naturally cross the blood-brain barrier. However, introduction of blood into the CSF during a traumatic lumbar puncture or defective permeability of the blood-brain barrier may lead to falsely elevated IgM levels in the CSF. Importantly, antibody cross-reactivity among the flaviviruses is not uncommon when using ELISA or IFA-based assays. The reference serologic method for arboviral antibody detection remains PRNT, which although technically challenging to perform and largely restricted to public health laboratories, provides higher specificity than routinely performed assays. Viral culture, while possible, is insensitive and not routinely offered at local or reference laboratories.

Table 73. Laboratory Diagnosis of Infection with West Nile Virus and Other Endemic Arboviruses in North America

Diagnostic Procedures	Optimal Specimens	Transport Issues
Serology	Serum	Clot or SST tube, RT, ≤2 h
NAAT[1]	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum	SST tube, RT, ≤2 h

[1] NAAT for uncommon arboviruses (e.g., California encephalitis viruses, LaCrosse encephalitis, St. Louis encephalitis virus, Eastern equine encephalitis virus, etc.) is available through the CDC or select public health laboratories.
PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Zika virus

Zika virus (ZIKV), a member of the *Flavivirus* genus and transmitted by *Aedes* spp mosquitos, has been causally linked to congenital birth defects, including microcephaly [489]. Diagnostic tests available for ZIKV include NAAT for viral RNA, serologic evaluation for IgM antibodies to the virus and PRNT, considered the reference standard for detection of neutralizing antibodies to arboviruses (Table 74). Selection between these methods (i.e., NAAT vs. serology) is primarily dependent on when the patient presents in relation to symptom onset or last possible exposure to

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ZIKV [490]. Currently, for symptomatic pregnant women who have traveled to areas with possible or active Zika transmission, the CDC recommends molecular testing by NAAT on serum and urine as soon as possible and up to 12 weeks after symptom onset. Additionally, they recommend testing by NAAT and IgM serology for dengue virus due to the similar geographic distribution and clinical presentation (https://www.cdc.gov/zika/hc-providers/testing-guidance.html). Importantly, Zika virus IgM testing is no longer recommended in symptomatic or asymptomatic pregnant women due to the persistence of this immunoglobulin for months to years after infection, limiting the ability to determine timing of infection, and due to the cross-reactivity with dengue virus IgM. For patients with a single Zika virus NAAT positive sample, the CDC recommends that the sample be re-extracted for re-testing to rule out the possibility of a false positive result. The CDC further recommends that non-pregnant patients, regardless of symptoms, should not be tested for Zika due to the very limited transmission of the virus currently; instead these individuals should be assessed for dengue virus. Finally, testing for Zika virus infections as part of preconception planning is not recommended.

Table 74. Laboratory Diagnosis of Zika Virus Infection[1]

Diagnostic Procedures	Optimum Specimens	Transport Issues
Serology	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Plasma	EDTA tube, RT, ≤2 h PPT tube, RT, ≤6 h
	Serum	SST tube, RT, ≤2 h
	Urine	Sterile, preservative-free container, RT, ≤24 h
	Whole blood	EDTA or citrate tube, RT, ≤2 h

[1] Additional specimens (e.g., products of conception, tissue, etc.) may be validated for testing at select public health laboratories or the CDC.
PPT, plasma preparation tube; RT, room temperature; SST, serum separator tube.

Lymphocytic choriomeningitis virus

Lymphocytic choriomeningitis virus (LCMV) is a rodent-borne virus that can cause meningoencephalitis and may be life-threatening in immunosuppressed persons. Serologic testing is the mainstay of diagnosis for LCMV infection and is typically established by demonstrating a four-fold or greater increase in IgG-class antibody titers between acute and convalescent phase serum samples, or by detection of anti-LCMV IgM antibodies (Table 75). Detection of antibodies in the CSF may indicate CNS infection; however, it may also be observed if the CSF fluid becomes contaminated with blood during collection, or if there is transfer of antibodies across the blood-brain barrier. NAAT can also be used to diagnose LCMV infection but is limited to select public health laboratories.

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Table 75. Laboratory Diagnosis of Lymphocytic Choriomeningitis Virus (LCMV) Infection

Diagnostic Procedures	Optimum Specimens	Transport Issues
Serology	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Serum	Clot or SST tube, RT, ≤2 h

RT, room temperature; SST, serum separator tube.

Monkeypox virus

Monkeypox virus (MPXV) is a member of the *Orthopoxvirus* genus, which includes variola virus (i.e., the causative agent of smallpox), vaccinia virus, camelpox and cowpox virus. Historically, monkeypox (mpox) outbreaks have been associated either with travel to the continent of Africa, or contact with animals (e.g., small rodents, certain non-human primates) that are indigenous to Africa. There are two phylogenetic clades of monkeypox virus; clade I (previously referred to as the central African [Congo Basin] clade), which is associated with higher morbidity and mortality (i.e., ~10% mortality rate) and clade II (previously referred as west African clade), which has an estimated mortality rate of ~3% [491]. Following exposure, infected individuals may develop a viral prodrome (i.e., fever, fatigue, myalgias) and subsequently an isolated or disseminated rash.

In 2022, the largest outbreak of mpox in recorded history occurred and was declared a public health emergency by the World Health Organization. In contrast to prior outbreaks, the 2022 outbreak was mainly associated with transmission through person-to-person contact, often involving sexual exposure [492]. In addition to sexual transmission, there were infrequent cases where transmission through contaminated fomites (i.e., bedding, clothing) was believed to be the source of infection.

MPXV can be readily cultured in a variety of eukaryotic cell lines, including primary rhesus monkey kidney cells. However, cultivation in viral cell culture represents a hazard to laboratory personnel and should not be performed for diagnostic purposes. Instead, testing of dermal and/or anogenital swab specimens from suspect lesions by molecular (i.e., real-time PCR) assays is the preferred approach for laboratory diagnosis [493]. If multiple lesions are present, 2 to 3 lesions should be collected and the swabs submitted either dry or in viral transport media to a testing laboratory (Table 76). Multiple lesions should be sampled due to the variable appearance of mpox lesions. The U.S. Centers for Disease Control and Prevention has an FDA-cleared non-variola *Orthopoxvirus* real-time PCR assay that can detect MPXV, while several commercially available MPXV-specific real-time PCR assay have received emergency use authorization from the FDA (https://www.fda.gov/medical-devices/emergency-use-authorizations-medical-devices/monkeypox-mpox-emergency-use-authorizations-medical-devices#molecular) .

Table 76. Laboratory Diagnosis of Monkeypox Virus Infection

Diagnostic Procedure	Optimum Specimens	Transport Issues

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NAAT	Dermal lesion swab	Sterile, preservative-free tube or in viral transport media, RT, ≤24 h
	Anogenital lesion swab	Sterile, preservative-free tube or in viral transport media, RT, ≤24 h

RT, room temperature.

Rabies virus

Rabies virus infects the central nervous system and is most often transmitted through the bite of a rabid animal. Diagnostic testing for rabies is not offered through most hospital or reference laboratories; therefore, consultation with a local public health laboratory or the CDC should be performed immediately in suspected rabies cases.

No single test is sufficient to diagnose rabies ante-mortem (Table 77). NAAT and viral isolation can be performed on saliva, immunohistochemistry may be performed on skin biopsies at the nape of the neck for detection of rabies antigen in the cutaneous nerves, and anti-rabies antibody testing is available for serum and CSF specimens. Post-mortem histopathology of brain biopsies in patients with rabies are notable for mononuclear infiltration, perivascular cuffing of lymphocytes, lymphocytic foci and Negri bodies. Serologic testing may be used to document post-vaccination seroconversion, if there is significant deviation from a prophylaxis schedule.

Table 77. Laboratory Diagnosis of Rabies Virus Infection

Diagnostic Procedure	Optimum Specimen	Transport Issues
Direct fluorescent antibody, Histopathology	Nuchal skin biopsy, Brain biopsy	Sterile, preservative-free container, RT, ≤24 h
Serology	Cerebrospinal fluid	Sterile, preservative-free tube, RT, ≤24 h
	Serum	Clot or SST tube, RT, ≤2 h
NAAT	Saliva	Sterile, preservative-free tube, RT, ≤24 h

RT, room temperature; SST, serum separator tube.

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Potential conflicts of interest

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