CHAPTER 65

MEDICAL PARASITOLOGY

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KEY POINTS

- Accurate diagnosis of parasitic infection usually depends on macroscopic or microscopic examination of specimens that have been appropriately collected and preserved. Thick and thin blood smears are useful for detecting and characterizing organisms found in the blood. Fecal specimens may be fresh (if they can be examined rapidly) or may be placed into fixatives such as formalin and polyvinyl alcohol or single-vial commercial fixatives. Examination of at least three specimens, collected on separate days, provides the optimal sensitivity for parasite detection. In general, it is not useful to examine specimens from hospitalized patients who develop diarrhea after the third hospital day since parasites are an unlikely cause of their symptoms.
- Antigen detection methods serve as a useful adjunctive or alternative test for detecting select parasites in clinical specimens. Immunoassays are commercially available for detecting *Plasmodium* spp. in blood; *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* in feces; and *Trichomonas vaginalis* in vaginal swabs.
- Serologic testing (i.e., detection of parasite-specific antibodies) is an important adjunct in recognizing parasitic infections that involve deep tissues, organs, and body spaces not readily amenable to routine morphologic testing methods.
- Molecular amplification methods generally offer high levels of sensitivity and specificity for diagnosis of parasitic disease. Most are laboratory developed tests (LDTs), although commercial options are available for some parasites, including multiplex panels that detect multiple gastrointestinal protozoan pathogens and nucleic acid amplification tests (NAATs) for *Trichomonas vaginalis*.
- Malaria is a protozoan parasite responsible for significant morbidity and mortality worldwide. The diagnosis of malaria should be considered in the differential diagnosis of unexplained fever with the history of travel in endemic geographic regions. Because of its high associated mortality, testing for acute disease should be done on a STAT basis. Thick and thin blood smears are complementary for detecting and identifying infecting *Plasmodium* spp., respectively, and are considered one of the gold standard laboratory methods. NAATs also offer high sensitivity and specificity but are rarely available on a STAT basis. Rapid antigen detection tests (RDTs) provide a quick presumptive diagnosis; however, they should be followed by more sensitive testing. Determination of parasite burden is used to direct initial therapy and monitor response to antimalarial treatment.
- Other important protozoal infections found in blood and/or tissue include babesiosis (blood), trypanosomiasis (blood, cerebral spinal fluid, and tissue), leishmaniasis (cutaneous, mucocutaneous, and visceral forms of disease), and toxoplasmosis. The latter often affects the central nervous system following congenital infection and in patients with acquired immunodeficiency syndrome (AIDS).
- Infection with intestinal amebae is acquired via ingestion of mature cysts, resulting in infection of the colon and passage of both cysts and trophozoites in the feces. Most amebae are nonpathogens. *Entamoeba histolytica* is a proven pathogen that may cause amebic dysentery, amebic colitis, and liver abscess. Diagnosis of amebiasis is made by microscopic examination of stool, antigen or nucleic acid testing of stool, and by serologic testing for antibodies in serum (for invasive disease).
- Flagellates include *Giardia duodenalis*, which causes diarrhea from ingestion of contaminated food or water and is diagnosed by the finding of trophozoites, cysts, and/or antigens in feces. *Trichomonas vaginalis* is acquired by sexual transmission and is detected in vaginal wet mounts by its characteristic motion or by more sensitive nucleic acid amplification techniques.
- *Cryptosporidium* and the coccidia (*Cystoisospora* and *Cyclospora*) can cause diarrhea in both immunocompetent and immunocompromised individuals. Symptoms are usually more protracted in immunocompromised individuals, such as those with AIDS. Special stains (e.g., modified acid-fast stain) are recommended for sensitive microscopic diagnosis; antigen and NAATs for *Cryptosporidium* spp. are also available and are more sensitive than microscopy.
- Helminths include nematodes (roundworms), cestodes (tapeworms), trematodes (flukes), and acanthocephalans (thorny-headed worms). These organisms reside as adults in the gastrointestinal tract or in other locations such as the liver, lung, and venous systems. Knowledge of their life cycles and zoogeography with intermediate hosts is important for understanding the clinical presentation and preventing transmission. Depending on the organism and its location(s) in the human host, eggs, larvae, or adult forms can be recovered from stool, urine, and/or sputum.
- Tissue helminths include filarial nematodes (adults in lymphatics, tissue and/or body cavities and larvae in blood or skin snips), *Trichinella* spp. (larvae are found in muscle), *Strongyloides stercoralis* (larvae in the lung, skin, and other organs during disseminated disease), and *Echinococcus* spp. (hydatidosis; cystic larval form in the liver, lungs, or other organs), among many others.
- Arthropods cause disease through direct tissue invasion, envenomation, vesication, blood loss, transmission of infectious agents, hypersensitivity reactions, and psychological manifestations. Characteristics necessary for identification can be maintained by preserving the organisms in alcohol (ticks, mites, fleas, lice, maggots) or by drying them (winged forms) after killing them with fumes of organic solvents.

TABLE 65.1
Taxonomic Relationships of the More Common Human Parasites

Modern Classification*	Representative Organisms
Protozoans	
Excavata: Metamonada (flagellates)	*Giardia duodenalis, Enteromonas hominis, Chilomastix mesnili, Retortamonas intestinalis, Dientamoeba fragilis, Trichomonas vaginalis, Pentatrichomonas hominis*
Excavata: Discicristata: Heterolobosea (free-living ameboflagellates)	*Naegleria fowleri*
Excavata: Discicristata: Euglenozoa: Kinetoplastea (hematoflagellates)	*Trypanosoma brucei, Trypanosoma cruzi, Leishmania* spp.
Amoebozoa: Discosea: Longamoebia (free-living amebae)	*Acanthamoeba* spp., *Balamuthia mandrillaris, Sappinia diploidea*
Amoebozoa: Archamoeba: Entamoebida (intestinal amebae)	*Entamoeba histolytica, E. dispar, E. moshkovskii, E. bangladeshi, E. coli, E. hartmanni, E. polecki, Endolimax nana, Iodamoeba buetschlii*
SAR: Apicomplexa: Conoidasida: Gregarinasina	*Cryptosporidium hominis, C. parvum*
SAR: Apicomplexa: Conoidasida: Coccidia (coccidians)	*Cystoisospora belli, Cyclospora cayetanensis, Toxoplasma gondii, Sarcocystis* spp.
SAR: Apicomplexa: Aconoidasida (malaria and babesiosis)	*Plasmodium falciparum, P. malariae, P. ovale, P. vivax, Babesia* spp.
SAR: Ciliophora (intestinal ciliates)	*Balantioides coli*
SAR: Stremenopiles (stremenopiles)	*Blastocystis* spp.
Trematodes	
Trematoda: Strigeatida (blood flukes)	*Schistosoma mansoni, S. haematobium, S. japonicum, S. mekongi, S. intercalatum, S. guineensis*
Trematoda: Echinostomida (intestinal and liver flukes)	*Echinostoma* spp., *Fasciola hepatica, F. gigantean, Fasciolopsis buski*
Trematoda: Opisthorchiida (small intestinal and liver flukes)	*Heterophyes heterophyes, Metagonimus yokogawai, Clonorchis sinensis, Opisthorchis* spp.
Trematoda: Plagiorchiida (lung and liver flukes)	*Paragonimus* spp., *Dicrocoelium dendriticum, Nanophyetus salmincola*
Cestodes	
Pseudophyllidea (pseudophyllidean cestodes)	*Dibothriocephalus latus, D. nihonkaiensis, Adenocephalus pacificus, Spirometra* spp.
Cyclophyllidea (cyclophyllidean cestodes)	*Taenia saginata, T. asiatica, T. solium, Echinococcus* spp., *Dipylidium caninum, Hymenolepis nana, H. diminuta*
Acanthocephalans	
Syndermata: Acanthocephala (acanthocephalans)	*Moniliformis moniliformis, Macracanthorhynchus hirudinaceus, M. ingens*
Nematodes	
Dorylaimia: Trichocephalida (trichuroid nematodes)	*Capillaria philippinensis, C. hepatica, Trichinella* spp., *Trichuris trichiura*
Chromadoria: Spururina: Ascarida (ascarid nematodes)	*Ascaris lumbricoides, Baylisascaris procyonis, Toxocara* spp., *Anisakis* spp., *Pseudoterranova* spp., *Contracaecum* spp.
Chromadoria: Spiruria: Oxyurida	*Enterobius vermicularis*
Chromadoria: Spiruria: Camallanida	*Dracunculus medinensis*
Chromadoria: Spiruria: Spirudida (spirudid and filarial nematodes)	*Brugia malayi, B. timori, Loa loa, Onchocerca volvulus, Wuchereria bancrofti, Mansonella perstans, M. ozzardi, M. streptocerca, Dirofilaria* spp., *Gnathostoma* spp., *Thelazia* spp.
Chromadoria: Rhabditina: Rhabditida (strongyles and hookworms)	*Strongyloides stercoralis, Ancylostoma duodenale, A. ceylanicum, Necator americanus, Angiostrongylus cantonensis, Trichostrongylus* spp.
Arthropods	
Crustacea: Maxillopoda: Pentastomida (tongueworms)	*Armillifer armatus, Linguatula serrata*
Arachnica: Acari (mites and ticks)	*Ixodes* spp., *Amblyomma* spp., *Dermacentor* spp., *Rhipicephalus* spp., *Hyalomma* spp., *Ornithodoros* spp., *Demodex* spp., *Sarcoptes scabiei*
Hexapoda: Insecta: Hemiptera (true bugs)	*Cimex lectularius, C. hemipterus, Triatoma* spp., *Rhodnius* spp., *Panstrongylus* spp.
Hexapoda: Insecta: Psocodea (lice)	*Pediculus humanus humanus, P. h. capitis, Pthirus pubis*
Hexapoda: Insecta: Siphonaptera (fleas)	*Ctenocephalides canis, C. felis, Pulex irritans, Xenopsylla cheopis, Tunga penetrans, T. trimamillata*
Hexapoda: Insecta: Diptera (myiasis-causing flies)	*Musca domestica, Phormia* spp., *Lucilia* spp., *Auchmeromyia senegalensis, Cochliomyia hominovorax, Chrysomya bezziana, Sarcophaga* spp., *Wohlfahrtia* spp., *Cuterebra* spp., *Dermatobia hominis, Cordylobia anthropophaga, Oestrus ovis*

Modified from Adl SM, Bass D, Lane CE, et al.: Revisions to the classification, nomenclature, and diversity of eukaryotes, *J Euk Microbiol* 66:4, 2019; and Adl SM, Mathison BA: Taxonomy and classification of human eukaryotic parasites. In: Carroll JC, Pfaller MA, Landry ML, et al., editors: *Manual of Clinical Microbiology*, ed 12, Washington, DC, 2019, ASM Press.

The study of parasitology has gained renewed importance in a world made smaller by the rapid movement of people and by the appearance of emerging and reemerging pathogens in immunocompromised individuals. Humans may be infected with a broad array of protozoan, helminthic, and arthropod parasites (Table 65.1). Various estimates have been put forth for the prevalence and related mortality figures of parasitic infections on a worldwide basis (Table 65.2). While the true incidence of many parasitic infections is unknown due to lack of public health reporting and limitations in clinical and laboratory expertise, it is clear that parasitic infections are an important cause of morbidity and mortality worldwide. Many important human parasitic diseases—such as malaria, leishmaniasis, trypanosomiasis, soil-transmitted helminthiases, and schistosomiasis—are concentrated in tropical and subtropical regions of the world, placing a tremendous burden on local health care resources while adversely affecting economic and societal development. Malaria, for example, was estimated by the World Health Organization (WHO) to infect 219 million people and cause 435,000 deaths in 2017 (WHO, 2018). Several additional parasitic infections have been classified as neglected tropical diseases (NTDs); combined, the NTDs are estimated to affect more than 1 billion people worldwide (WHO, 2019). Other parasitic diseases—such as scabies, giardiasis, enterobiasis (pinworm infection), toxoplasmosis, trichomoniasis, and pediculosis—have a broader distribution and may be found in temperate regions of the United States, Canada, and Europe. The United States Centers for Disease Control and Prevention (CDC) has targeted five neglected parasitic infections (NPIs) for public health action based on the number of individuals affected, the severity of infection, and the availability of adequate treatment and prevention modalities (CDC, 2018b). Regardless of geographic location, the ease and rapidity of global travel requires that clinicians and laboratorians be familiar with the array of important human parasites and their means of diagnosis.

This chapter provides an overview of the general approach used by laboratorians to recover and identify parasitic protozoa and helminths from human clinical specimens. Discussion of individual species of parasites focuses on essential clinical and biological information necessary to assist in diagnosis and management. For more extensive coverage of specific parasites, a number of excellent texts are available (Beaver et al., 1984; John & Petri, 2006; Ryan et al., 2019; Farrar et al., 2013; Roberts et al., 2013; Garcia, 2016; Mullen & Durden, 2018; among others). Some of these references are older and may be less accessible; however, they discuss classic disease presentations and historic perspectives in a way that is sometimes lacking in newer literature. Parasitology atlases are also important resources for any laboratorian performing parasitology examinations and should be readily available (Ash & Orihel, 2007; Pritt, 2014; Mathison & Pritt, 2015). Several texts specifically address the pathologic aspects of parasitic infections (Orihel & Ash, 1995; Meyers, 2000; Meyers et al., 2013; Pritt, 2018). The DPDx website hosted by the CDC (https://www.cdc.gov/dpdx/index.html) presents life cycles, epidemiology, clinical presentation, diagnostic methodologies, and image galleries for human parasitic infections. They also offer a telediagnostic service (https://www.cdc.gov/dpdx/contact.html) for medical and public health professionals.

PARASITE CLASSIFICATION AND TAXONOMY

Parasites are traditionally placed into one of three groups: the helminths (worms), protozoans, and arthropods. Historically, parasites within each group are then classified within hierarchical levels (i.e., Phylum, Class, Order, Family, and so on) based primarily on morphologic features and phenology (life cycle events and their relation to environmental factors). Unfortunately, the conventional classification scheme is somewhat subjective and does not always reflect phylogenetic relatedness between organisms within each grouping. Recent advances in biochemical and molecular methods, including whole-genome sequencing, have led to a revision of the classification of protozoans using a system based on hierarchical ranks (e.g., Super-group, First Rank, Second Rank) that reflect phylogenetic relatedness (Adl et al., 2012; Adl et al., 2019). Many of the familiar grouping names, such as *Apicomplexa*, *Entamoebidae*, and *Trypanosomatidae*—have been retained for ease of communication, but they are no longer linked to level descriptors such as *Order*, *Class*, and so on. However, this new classification is complex and used primarily in the research setting and by protozoologists studying nonparasitic organisms. While this system is gaining acceptance in the medical field for protozoa, the classic Linnaean hierarchal system of taxonomy is still commonly used for helminths and arthropods. An updated version of the traditional schemata incorporating the revised protozoan classification is presented in this chapter (Adl & Mathison, 2019).

LABORATORY METHODS

Numerous methods have been described for the recovery and identification of parasites in clinical specimens, some of which are useful for detection of a variety of organisms, whereas others detect only a particular species. It is preferable for the laboratory to offer a limited number of procedures that can be competently performed rather than a larger variety of infrequently performed tests for which competency cannot be reliably maintained provided that the laboratory is able to meet the clinical needs of the population it serves. At a minimum, most laboratories should be able to provide rapid detection of life-threatening parasitic infections such as malaria and primary amebic meningoencephalitis. Tests for detecting less serious infections may be sent to a reference laboratory if clinically applicable. As newer information becomes available on certain so-called emerging parasites, the laboratory may need to develop and use additional highly specific test methods or find competent referral laboratories where such tests are performed.

The types of specimens collected for laboratory evaluation depend on the species and stage of the parasite suspected. Knowledge of the life cycle of the parasite aids in determining the type, number, and frequency of specimens required for diagnosis. Analyses of blood and fecal specimens account for the largest share of clinician requests for parasitologic evaluation. A variety of additional specimens is submitted to the laboratory less frequently, including urogenital specimens, sputum, aspirates, and biopsy material. In addition to traditional microscopic examination, immunologic and molecular methods are useful in many instances and may be the only methods available in certain circumstances. Complete descriptions of general and esoteric laboratory procedures for the recovery and identification of parasites referred to here may be found in a variety of sources to which the reader is referred (Beaver et al., 1984; Ash & Orihel, 1987, 2007; Garcia, 2009, 2010, 2016; Garcia et al., 2018; Carroll et al., 2019). This chapter will focus on those that are most relevant for routine clinical use.

TABLE 65.2
Estimated Prevalence of Parasitic Infections Worldwide

Disease	Estimated Global Population Involved	Estimated Annual Number of Deaths
Protozoan		
Amebiasis	28 million illnesses/year	1470
African trypanosomiasis	<15,000 currently infected	NA
American trypanosomiasis (Chagas disease)	8 million currently infected	10,000
Cryptosporidiosis	8.5 million illnesses/year	3759
Echinococcosis	>1 million	19,300
Giardiasis	28 million illnesses/year	0
Leishmaniasis	1 million cutaneous illnesses/past 5 years; 300,000 cases of visceral leishmaniasis/year	26,000–65,000
Malaria	124–283 million illnesses/year	367,000–755,000
Toxoplasmosis	10.3 million illnesses/year	684
Helminthic		
Cestodiases (including cysticercosis)	340,864 illnesses/year	36,500
Clonorchiasis/opisthorchiasis	47,935 illnesses/year	7268
Dracunculiasis	28 illnesses/year	0
Fascioliasis	10,635 illnesses/year	0
Intestinal trematodiases	18,924 illnesses/year	0
Lymphatic filariasis	120 million	NA
Neurocysticercosis	2.56–8.30 million currently infected	NA
Soil-transmitted helminthiases (primarily hookworm infection, ascariasis, trichuriasis)	1.5 billion	>1000
Onchocerciasis	20.9 million	0
Paragonimiasis	139,238	250
Schistosomiasis	>166 million	200,000
Strongyloidiasis	30–100 million	NA
Trichinosis	4472 illnesses/year	4

Data from the Centers for Disease Control and Prevention, World Health Organization (WHO, 2015, 2019), and the GBD (GBD, 2017).
NA, Not available.

Familiarity with calibration and use of the ocular micrometer is necessary for any laboratory performing parasitologic examination, as measurement of the size of protozoal trophozoites and cysts and of helminth eggs and larvae is often required to make an accurate identification. For example, size is an important consideration for differentiating some of the intestinal protozoa (e.g., *Entamoeba histolytica* vs. *Entamoeba hartmanni*, *Cryptosporidium* spp. vs. *Cyclospora cayetanensis* oocysts), and morphologically similar helminth eggs (Ash & Orihel, 2007; Pritt, 2014).

EXAMINATION OF BLOOD

Parasites that may be detected in blood specimens include the agents of malaria (*Plasmodium* spp.), babesiosis (*Babesia* spp.), trypanosomiasis (*Trypanosoma* spp.), leishmaniasis (*Leishmania* spp.), and filariasis (*Wuchereria bancrofti*, *Brugia* spp., *Loa*, and *Mansonella* spp.). The most important techniques to be performed in the clinical laboratory to assist in the diagnosis of blood parasites include preparation, staining, and examination of thick and thin blood films. Other techniques used less frequently include the buffy coat smear (useful for detecting trypanosomes) and various concentration techniques reserved for recovery of microfilariae (WHO, 1987; Ash & Orihel, 2007; Garcia, 2016).

Thick and Thin Blood Films

Examination of permanently stained blood films is required to identify most blood parasites (Clinical and Laboratory Standards Institute [CLSI], 2000). Thin films are prepared in the same manner as for hematologic differential evaluation; blood is spread over the slide in a thin layer, yielding intact, nonoverlapping cellular elements (Fig. 65.1). Integrity of the blood cell membranes is important for determining the intracellular or extracellular nature of the infection and the size of the infected erythrocyte. In the thick film, blood is concentrated in a small area that is many cell layers deep. During staining, erythrocytes are dehemoglobinized and only leukocyte nuclei, platelets, and parasites (if present) are visible. The thick film is preferred for parasite *detection* because it contains 15 to 30 times more blood per microscopic field than does the thin film, thus increasing the chances of detecting light parasitemia and decreasing the time needed for reliable examination (Pritt, 2019). Although examination of thick films increases the likelihood of detecting an infection, species identification is usually performed by examination of thin films because morphology is often more definitive, especially for malarial parasites. For routine detection of malaria and babesiosis, both thick and thin films should be prepared (CLSI, 2000).

Preparation of Slides

Blood for examination may be obtained by fingerstick, earlobe puncture, or venipuncture. Fingerstick blood should flow freely to prevent dilution with tissue fluid, and it should not be contaminated with the alcohol disinfectant, which should be allowed to dry first. If obtained by venipuncture, the first drop of blood (anticoagulant-free) from the needle is used to prepare the films at the bedside (CLSI, 2000). Use of anticoagulants is discouraged when malaria is suspected because they may cause distortion of the parasites and interfere with staining. In practice, however, blood usually is submitted to the laboratory in an anticoagulant, which may be the only practical method to ensure that high-quality smears can be prepared. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood is preferred in such cases; the specimen should be transported to the laboratory within the hour to prevent deterioration of organism morphology (CLSI, 2000). Anticoagulants do not interfere with the staining of microfilariae.

Both thin and thick films should be prepared on clean, grease-free slides. Thick films are prepared by placing 1 to 2 small drops of blood onto a slide and spreading them into an area the size of a dime (1.5 cm) with the edge of a second slide (CLSI, 2000; Pritt, 2019). The blood film is then allowed to dry flat at room temperature. Drying time may be decreased by placing the slides in a laminar flow hood. A proper thick film should be thin enough that newspaper print may be faintly readable through it (CLSI, 2000). If it is too thick, the film may peel from the slide. Adherence can be greatly improved by gently pushing (grinding) down with the corner of the second slide while spreading the droplet, creating minute scratches on the carrier slide that provide additional surface area for the blood film (see Fig. 65.1) (Norgan et al., 2013).

This method does not affect the microscopic morphology and allows the film to be stained as soon as it is dry (within 30–60 minutes). Heating the slides to decrease drying time is discouraged since excess heat may fix erythrocytes and prevent them from lysing when placed in the stain reagents (CLSI, 2000).

Staining

Blood begins to lose its affinity for stain in about 3 days, and the erythrocytes in older thick films do not lyse well. Therefore, slides stored for archival purposes should first be stained. Best staining results are achieved when using Giemsa stain, because host cell and parasite chromatin stains vividly while the hemoglobin in the erythrocytes is only a pale purple-red. When used at a neutral pH (7.0–7.2), this method also allows for optimal visualization of erythrocyte inclusions (e.g., Schüffner stippling, Maurer clefts) that occur with infection by certain malarial parasites (Pritt, 2019). Wright and Wright-Giemsa stains may also be used for thin films (as is common in the hematology lab), but they stain parasites less well than Giemsa and do not allow for visualization of erythrocyte inclusions due to the lower pH (<6.8). Because these stains incorporate alcohol as their fixative, thick films must be lysed in water before staining (CLSI, 2000; Pritt, 2019).

The Giemsa staining procedure requires somewhat more attention to preparation of reagents and staining protocol than does the Wright staining procedures, which are often automated. Generally, fresh Giemsa stain must be made each day of use by diluting stock solution into phosphate-buffered water (Garcia, 2016). Each new lot of stock Giemsa stain must be checked to determine optimal staining time and dilution because some variation is seen from lot to lot (Garcia, 2016).

Examination of Smears

Both thick and thin smears are examined in their entirety under the low-power (10×) objective to detect microfilariae, which rarely occur in large numbers. In particular, the feathered edge of thin smears should be examined, as microfilariae are often carried there during preparation of the smear (CLSI, 2000). Examination using a 50× oil immersion objective may subsequently be used to screen blood films for protozoa, although thorough examination using the 100× oil immersion objectives still is necessary to detect the smallest parasites, such as *Plasmodium* and *Babesia* spp. The optimal location for examining the thin film is the region of the feathered edge where there is minimal overlap of cells and the erythrocytes maintain their central pallor (Pritt, 2019). A common mistake is to examine regions of the thin film where the blood is too thick or too thin and the parasite morphology is distorted. An experienced microscopist should examine at least 100 oil immersion fields on both the thick and thin blood film and up to at least 300 fields for immunologically naïve patients who might present with more severe symptoms at a lower parasitemia (Mathison & Pritt, 2017).

Blood Concentration Techniques

A variety of special techniques have been described for the concentration of blood parasites—specifically, leishmaniasis, trypanosomes, and microfilariae—details of which may be found elsewhere (Beaver et al., 1984; CLSI, 2000; Ash & Orihel, 2007; Garcia, 2009, 2016).

Preparation of buffy coat smears, which most clinical laboratories can perform with existing resources, is helpful in the detection of *L. donovani*, trypanosomes, and microfilariae (CLSI, 2000). Following centrifugation of an anticoagulated blood sample, the layer of cells between plasma and packed erythrocytes is drawn off and used to prepare blood films for staining or for preparation of a wet mount to detect motile organisms (Ash & Orihel, 2007).

For detection of microfilariae, the Knott concentration technique or membrane filtration is helpful, particularly when the density of

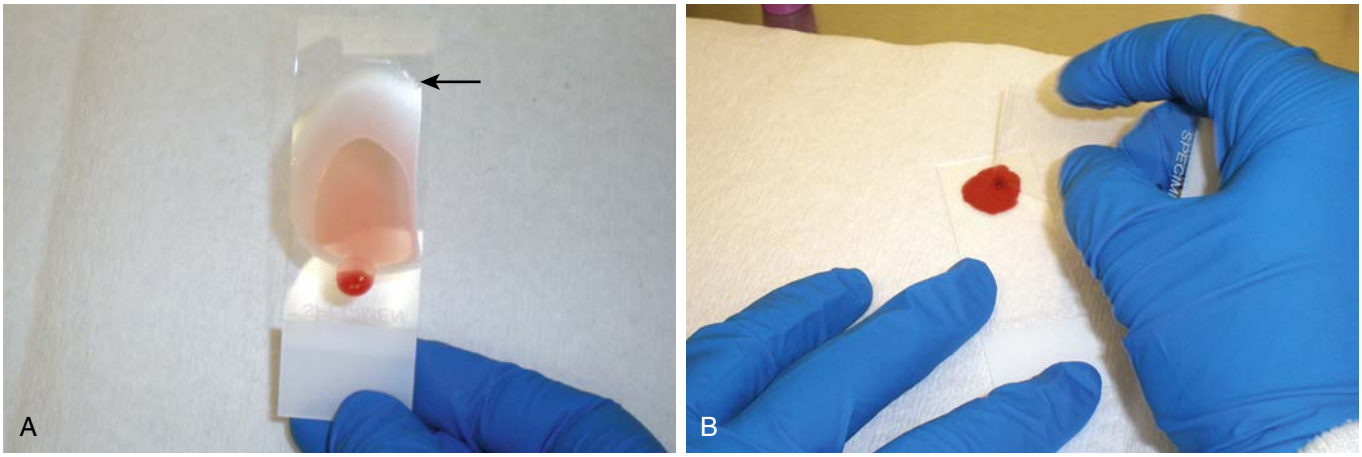


Figure 65.1 **A,** Thin blood film demonstrating a broad feathered edge *(arrow)*. **B,** Preparation of a thick blood film. Gentle pressure is used to create minute scratches in the carrier slide while spreading the blood into a 1.5- to 2.0-cm diameter film with the spreader slide. This method improves the adherence of the thick film to the carrier slide and prevents it from detaching during staining.

microfilariae in peripheral blood is very low. With the Knott concentration technique, anticoagulated blood is lysed with 2% formalin and centrifuged to concentrate the microfilariae in the sediment, which then may be examined as a wet preparation or stained with Giemsa or hematoxylin stain. In the membrane filtration procedure, blood is lysed and passed through a 5-μm membrane filter, which is subsequently stained with hematoxylin to reveal any microfilariae (WHO, 1987; CLSI, 2000; Ash & Orihel, 2007; Mathison et al., 2019).

Finally, use of the fluorochrome acridine orange in a microhematocrit centrifuge format (QBC Malaria test; Drucker Diagnostics, Port Matilda, PA) allows detection of *Plasmodium* spp. and other blood parasites. While it appears to be more sensitive than traditional thick and thin smears, it is not widely used due to the need for a fluorescent microscope (CLSI, 2000). Fluorescent attachments to traditional light microscopes may make this test more widely accessible. In positive cases of malaria or babesiosis, traditional blood films must be examined for *Plasmodium* spp. identification (if relevant) and calculation of parasite burden.

EXAMINATION OF FECAL SPECIMENS

The presence of intestinal parasites is primarily identified through the direct examination of feces using wet mounts, concentration techniques, and permanently stained smears. Microscopic examination of feces is commonly referred to as the "ova and parasite" exam, or O&P. Stages of helminths commonly recovered include eggs and larvae, although intact worms or portions thereof may occasionally be identified by gross examination. Intestinal protozoan infections are diagnosed by detection of trophozoites, cysts, or oocysts. Routine methods should include procedures that permit recovery of both protozoa and helminths, with the use of special procedures (e.g., stains for coccidians) limited to specific requests. Ideally, laboratories performing parasitologic examination should be capable of performing a concentration procedure and a permanent stain method, as many protozoan infections will be missed unless permanent stains are examined (Ash & Orihel 2007; Garcia, 2009, 2010, 2016; Garcia et al., 2018).

Specimen Collection, Handling, and Preservation

Recovery and subsequent identification of parasites in fecal specimens requires proper collection and handling. Old, poorly preserved, or contaminated specimens are of little value. Additionally, specimens should not be collected for 1 week after the patient has ingested any materials that leave a crystalline residue, such as nonabsorbable antidiarrheal compounds, antacids, bismuth, barium, or antimalarial agents. Oily laxatives such as mineral oil may also interfere with examination (CLSI, 2005). Use of antibiotics or contrast media may decrease the numbers of organisms, especially protozoa, in the intestinal tract for several weeks (Ash & Orihel, 2007; Garcia, 2009, 2016; Garcia et al., 2018).

Specimens may be submitted to the laboratory fresh or in appropriate preservatives. Fresh specimens should ideally be examined shortly after passage, with liquid specimens being examined within 30 minutes of passage, whereas semi-formed specimens should be examined within 1 hour, and solid specimens within 24 hours of passage (Garcia et al., 2018). Given that most labs cannot accommodate these time frames, it is best to place the specimen immediately into a suitable preservative to maintain parasite morphology and ensure that fragile protozoal trophozoites are not inadvertently destroyed (Garcia et al., 2018). Unpreserved specimens should be refrigerated if they cannot be examined immediately. Specimens in preservative can be stored at room temperature.

Specimens may be passed directly into clean, dry containers, or onto a specially designed wax or plastic collection sheet that is placed over the toilet bowl (CLSI, 2005; Garcia et al., 2018). Diarrheic specimens may also be collected in clean bedpans. Containers should have tight-fitting lids and should be placed in plastic bags before transport to the laboratory. Inadvertent introduction of urine or toilet water with the specimen may readily destroy protozoal trophozoites and should be avoided. Also, contamination with water or soil may accidentally introduce free-living organisms that may prove difficult to differentiate from parasitic ones (Garcia et al., 2018).

Kits consisting of vials of preservatives appropriate for performing direct examinations, concentration procedures, and preparation of stained smears are available from a number of commercial sources at relatively low cost. Aliquots of freshly passed stool should be immediately placed into these vials and mixed thoroughly. These kits are especially helpful for those patients who are unable to bring in a fresh sample in timely fashion or for those who will be collecting several specimens over the course of several days. With the classic two-vial technique, one portion of specimen is fixed in three parts of 5% to 10% buffered formalin and another portion in three parts of polyvinyl alcohol (PVA) fixative. Other available preservation systems include merthiolate-iodine-formalin (MIF) and sodium acetate–formalin (SAF; Table 65.3). SAF has an advantage in that it can be used for permanent stains as well as for direct mounts and concentration procedures and it does not contain mercury, which is present in Schaudinn and PVA fixatives. In addition to being poisonous, mercury presents disposal problems in an increasing number of states. However, the quality of permanent stains when SAF is used is not as good as when Schaudinn or PVA fixative is used. Zinc sulfate–based PVA and other newer commercial products such as single-vial multipurpose fixatives are gaining popularity, and their use may be indicated when mercury chloride–based compounds cannot be used (Garcia et al., 2018; Carroll et al., 2019).

Examination of three specimens collected on different days over a maximum 10-day period is considered the minimum necessary to perform an adequate O&P evaluation (Garcia, 2016; Garcia et al., 2018; Carroll et al., 2019). This procedure ensures an optimum interval for recovery of those parasites known to shed diagnostic forms intermittently. However, for certain parasites, such *Strongyloides stercoralis*, up to seven O&P examinations may be necessary for optimal detection. Additional sensitivity may be achieved in detecting these parasites, as well as *E. histolytica*, using antigen detection methods, nucleic acid amplification tests (NAATs) and concentration techniques (discussed later). In general, it is not useful to examine specimens from hospitalized patients who develop diarrhea after the third

TABLE 65.3

Commonly Used Stool Fixatives and Examination Techniques and Whether Fixatives are Appropriate for Antigen Detection or Molecular Assays

	EXAMINATION TECHNIQUE				
Fixative	**Direct Wet Mount**	**Wet Mount Concentration**	**Permanent Stained Smear**	**Antigen Detection**	**NAATs***
None (fresh stool)	Yes	Yes	Yes	Yes	Yes
10% formalin	Yes	Yes	No	Some	No
Schaudinn fluid	No	No	Yes	No	No
Polyvinyl alcohol (PVA)	No	No	Yes	No	No
Modified PVA†	No	Yes	Yes	No	Some
Merthiolate-iodine-formalin (MIF)	Yes	Yes	No‡	No	No
Sodium acetate–formalin (SAF)	Yes	Yes	Yes	No	No
Single-vial systems**	Yes	Yes	Yes	Some	Some

*NAATs: Nucleic acid amplification tests.
**Many commercially available single-vial fixatives are now available, for example, TOTAL-FIX (Medical Chemical Corporation, Torrance, CA), Para-Pak EcoFIX (Meridian Bioscience, Cincinnati, OH), ALCORFIX and APAFIX (Apacor, Berkshire, England), and may be used for both the concentration procedure and permanent stained smear as well as antigen detection and NAATs.
†Copper sulfate or zinc sulfate replaces the mercuric chloride.
‡Smears prepared from MIF-preserved specimens may be stained with polychrome IV stain.

hospital day since parasites are an unlikely cause of their symptoms. Once the specimens are received in the laboratory, they should undergo macroscopic and microscopic examination as detailed next.

Macroscopic Examination

Fecal specimens should be examined grossly for consistency (formed, soft, loose, or watery) and for the presence of mucus, blood, adult worms, and proglottids. Protozoan trophozoites are more likely to be found in watery or loose specimens, whereas cysts predominate in formed or soft specimens. Helminths or their eggs may be found in any type of fecal specimen. Most parasites are uniformly distributed in the stool as a result of the mixing action of the cecum, although some eggs (especially schistosomes) may enter the fecal stream in the lower colon and rectum and may be unevenly distributed, as may pinworm and *Taenia* spp. eggs. Protozoan trophozoites may be more numerous in the last portion of stool evacuated and should be specifically sought in mucus (Garcia, 2016).

Microscopic Examination

Specimens may be examined microscopically by direct wet mounts of fresh or preserved material, wet mounts of concentrated feces, or permanent stains. Each procedure has specific advantages and limitations, and not all may be routinely used in the clinical laboratory. Direct saline wet mounts of fresh feces allow detection and observation of motile protozoan trophozoites and helminth larvae. Direct mounts of preserved feces may allow detection of parasites that do not concentrate well. Concentration procedures increase the examiner's ability to detect protozoan cysts and helminth eggs and larvae but are unsatisfactory for detecting protozoan trophozoites. Permanent stains are useful for detection and morphologic examination of protozoan trophozoites and cysts.

The circumstances under which each procedure is performed vary depending on the workflow of the laboratory. As mentioned earlier, examination of unpreserved specimens allows for enhanced detection of motile parasites. Many protozoa can also be differentiated by their characteristic motility patterns. However, examination of unpreserved stool is useful only when specimens can be examined shortly after being passed. The direct wet mount may be omitted if the specimen is submitted in a preservative. At a minimum, fixed specimens should be examined by a concentration procedure, which provides increased sensitivity for detection of parasites. However, improved yield has been demonstrated when a permanent stain is also examined, as the two preparations are complementary. Therefore, the optimal stool examination includes both a concentrated wet prep and a permanently stained preparation (CLSI, 2005, Garcia, 2010, 2016; Garcia et al., 2018). The following provides detailed instructions for performing the direct wet mount, concentration techniques, and permanently stained preparations.

Direct Wet Mount

The direct wet mount is one of the most easily performed parasitologic tests, although proper interpretation requires careful examination and experience in using the microscope to full advantage. The test is most useful when fresh specimens, especially liquid stools or duodenal aspirates, are examined for motile trophozoites or helminth larvae, although it can also be performed using fixed specimens. A small amount of stool is mixed with a drop of 0.85% saline and covered with a coverslip.

Examination of the entire coverslip is performed systematically under the low-power (10×) objective, with the microscope diaphragm closed down to increase contrast. Suspicious objects and those that are refractile, such as protozoal cysts, should then be examined with the high-power (40×) objective. Detection of motility of slow-moving amebae requires that an object be examined for at least 15 seconds. In the absence of suspicious objects, up to a third of the preparation should be examined using the 40× objective. The oil immersion objective usually is not used unless the coverslip has been sealed with nail polish or Valspar (a 50:50 mixture of petroleum jelly and paraffin).

A second preparation may be made in identical fashion, except that a drop of a 1:5 dilution of Lugol iodine or an equivalent preparation is added in place of the saline. Use of straight Lugol or iodine causes clumping of material and is not recommended. Iodine is helpful in enhancing the visibility of nuclear structures in protozoal cysts and in detecting glycogen inclusions. Limitations, however, include loss of trophozoite motility and cyst refractivity, as well as difficulty in recognizing chromatoid bodies.

Concentration Techniques

Concentration procedures, which may be performed on fresh or preserved specimens (see Table 65.3), are more sensitive than direct wet mount examination for detection of protozoan cysts and helminth eggs and larvae because they decrease the amount of background material in the preparations and, in most circumstances, actually concentrate the organisms. A wet mount of the concentrated specimen is examined as described earlier for the direct wet mount. Although a variety of methods and modifications have been described, some are useful only for specific parasites (Ash & Orihel, 1987; Garcia, 2010, 2016; Garcia et al., 2018). For routine use, a method should be selected that allows reliable detection of both protozoan cysts and helminth eggs. Concentration methods are based on sedimentation or flotation principles. In sedimentation, the parasites settle to the bottom as a result of gravity or centrifugation. In flotation, the parasite cysts and eggs rise to the surface of a solution of high specific gravity. Flotation is less commonly used in the United States for human clinical specimens but is commonly used in veterinary medicine. Traditionally, concentration methods involved one or two-step processes involving formalin and ether or ethyl acetate. However, single-vial systems allow for concentration of fecal specimens, often by using less hazardous chemicals (Couturier et al., 2015).

The traditional formalin–ethyl acetate concentration is a biphasic sedimentation technique that is efficient in recovering most protozoan cysts and helminth eggs and larvae, including operculate eggs, and is moderately effective for schistosome eggs. Less distortion of protozoal cysts occurs with this technique than with zinc sulfate flotation. For proper concentration of coccidian oocysts, attention must be paid to the recommended speed and time of centrifugation (Garcia, 2010, 2016; Garcia et al., 2018). Despite these problems, the technique is used widely for both its simplicity and its suitability in most laboratory situations.

With the zinc sulfate flotation method, fresh stool is processed using zinc sulfate with a specific gravity of 1.18, and formalinized stool is processed with a solution of specific gravity of 1.20. Parasitic elements are recovered from the surface film of the solution following centrifugation. This method yields a cleaner preparation than is provided by formalin–ethyl acetate concentration, but it is unreliable for the recovery of nematode larvae, infertile eggs of *Ascaris*, and the eggs of most trematodes and large tapeworms. Problems with recovery also occur with stool specimens containing excessive amounts of fats. Use of formalinized stool specimens rather than fresh stool helps clear the specimen and prevents popping of opercula and distortion of the parasites (Bartlett et al., 1978).

Permanent Stains

Use of stained slide preparations provides a permanent record of a patient's specimen and allows review by consultants should difficulties arise in identification. These preparations are also amenable to whole-slide scanning for production of a digital image. Of the methods described for studying fecal specimens, only the permanent stain is designed for analysis using the oil immersion objective (100×). The permanent stain is most useful for detection of protozoal trophozoites and cysts, which may be recognized when direct and concentrated preparations are negative. Although they generally are not useful for detecting helminth eggs or larvae, permanent stains are inherently more sensitive for detecting protozoal infections, and their use has been recommended for every stool sample submitted for O&P examination (Garcia, 2016; Garcia et al., 2018).

A variety of staining techniques and modifications and their advantages and disadvantages have been described. The Wheatley trichrome stain and iron-hematoxylin stain are all-purpose methods that allow detection of amebae and flagellates. Unfortunately, most coccidia are not readily detected by these stains; thus, additional special stains (see next sections) must be employed. Technical problems may arise in the performance of any staining procedure; most are related to the age of the specimen, proper smear preparation and fixation, and the quality of the reagents. Positive control slides of known staining quality should be run with each batch of slides stained or, at the very least, once daily after reagents are changed. This is especially true in the performance of more specific stains for the coccidia. Less commonly used stains, such as polychrome IV stain for use with MIF-preserved specimens and chlorazol black E stain for use with fresh specimens, are not reviewed here. Details may be found elsewhere (Garcia, 2016; Garcia et al., 2018).

Wheatley Trichrome Stain. In the United States, the Wheatley modification of the trichrome method continues to find widespread acceptance because of its simplicity, reliability, and cost-effectiveness. Details of the procedure are available from a number of sources (Ash & Orihel, 2007; Garcia, 2010, 2016; Garcia et al., 2018). Appropriate specimens include those that have been fixed in Schaudinn fixative or PVA fixative; SAF- or MIF-preserved specimens may be stained with trichrome, but results are less satisfactory. Specimens preserved using single-vial commercial fixatives may also be stained with this or a slightly modified protocol. For example, EcoStain (Meridian Bioscience, Cincinnati, OH) is a modified trichrome stain that works well with non–mercury-based fixatives.

Iron Hematoxylin Stain. The traditional iron hematoxylin stains are technically more difficult to perform than the trichrome stain; thus, they are becoming increasingly rare in the United States. However, results generally are superior owing to enhanced definition of key nuclear and cytoplasmic characteristics, and most of the original descriptions of protozoan morphology are based on slides stained by these methods. A modified iron hematoxylin stain that incorporates carbol fuchsin has been described, which allows concurrent staining of acid-fast organisms such as *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* (Garcia, 2010, 2016; Garcia et al., 2018). Specimens fixed in Schaudinn, PVA, or SAF fixative may be stained with iron hematoxylin stains (the preferred stain for SAF).

Modified Acid-Fast Stains. Oocysts of *Cryptosporidium*, *Cyclospora*, and *Cystoisospora* are difficult to recognize on concentrated wet preparations, trichrome- or iron hematoxylin–stained smears, but their presence may be detected by using an acid-fast staining technique such as the modified Kinyoun method, modified acid-fast dimethyl sulfoxide, or auramine-O or staining with safranin (hot method) (Cama & Mathison, 2015). Acid-fast stains are sensitive and cost-effective for detection of these protozoa, but they lack specificity. Close attention must be paid to defined morphologic criteria when these stains are used, and the use of positive control material is mandatory. For laboratories in which *Cryptosporidium* is rarely encountered, use of the highly specific and sensitive commercially available immunoassay reagents is recommended. Stool, sputa, biliary tract, and other appropriate specimens that are fresh, formalin fixed, or SAF fixed may be used with acid-fast stains.

Additional Techniques for Examination of Enteric Parasites

Cellulose Tape and Paddle Techniques for Pinworm

The female pinworm, *Enterobius vermicularis*, migrates from the cecum to the perianal skin, where she deposits typical eggs that are partially embryonated. The eggs or, occasionally, adult worms may be detected on examination of clear, adhesive cellophane tape or commercial collection kits that have been pressed on to the perianal skin (Fig. 65.2).

Eggs or adults are not commonly found in stool concentrates, which is considered to be an inappropriate specimen for detection of this parasite. Specimens should be collected first thing in the morning before bathing or defecation. Several specimens taken on different days should be examined before infection is ruled out. Commercial devices such as the SWUBE (Becton Dickinson, Franklin Lakes, NJ) use an adhesive paddle for collection and greatly simplify specimen collection and examination.

Egg Studies

Estimations of worm burden were historically requested to assist in the evaluation of therapeutic efficacy, identify patients with heavy infections that warrant anthelminthic therapy, or for following rates of reinfection with intestinal nematodes (*Ascaris*, *Trichuris*, and hookworms) or, occasionally, schistosomes. Quantification methods are less commonly used today in resource-rich settings since it is standard practice for all infected patients to be treated, regardless of the egg burden, and the currently available drugs are highly efficacious. Procedures for egg quantification include the direct smear method of Beaver, the Stoll dilution egg count, Kato thick smear, and various modifications (Beaver et al., 1984; Ash & Orihel, 1987, 2007). Large variations in results are inherent when these tests are performed, and levels of egg counts indicating clinical significance vary, depending on the infecting species and the person's age and nutritional status (Beaver et al., 1984).

Nematode Culture and Recovery Techniques

Several culture techniques (coproculture) assist in the detection and identification of certain nematode infections, including the Harada-Mori filter paper strip culture, filter paper/slant culture, and charcoal culture (Beaver et al., 1984; Ash & Orihel, 1987, 2007; Garcia, 2016; Garcia et al., 2018). Differentiation of hookworms and trichostrongyles on the basis of egg morphology is difficult, whereas infective-stage larvae are more readily identified. Such culture techniques may also prove useful in recovery of *Strongyloides* larvae, which may be few in number, and in differentiating them from those of hookworms. With all culture methods, feces are incubated in a humid environment to encourage egg hatching. With the Harada-Mori and filter paper/slant techniques, larvae migrate from the feces into a water phase, where they may be readily detected. In the charcoal culture, larvae first migrate into a dampened gauze pad, which is then placed in water, allowing the larvae to settle out. These methods are most commonly used in clinical laboratories in endemic settings.

The Baermann funnel technique and agar culture methods are sensitive and reliable methods for recovery of *Strongyloides* and other nematode larvae from a stool specimen. In the Baermann assay, feces are placed on several layers of gauze on top of a wire screen that is suspended in a funnel. The bottom of the funnel is clamped off, and water is added to the level of the gauze. Larvae actively migrate through the gauze and settle to the bottom of the funnel, where they may be drawn off for examination. Although this method provides increased sensitivity over the traditional O&P exam, it is labor-intensive and used infrequently in the clinical laboratory. The agar culture technique provides a simpler and more sensitive means for detecting *S. stercoralis* in feces. With this method, feces are plated on a nutrient agar and incubated at room temperature for several days. Over time, the larvae will migrate out of the feces into the agar and carry fecal bacteria with them. Growth of the bacteria in the larval tracks facilitates identification of larvae in the specimen (Fig. 65.3).

In latent *Strongyloides* infection, in which few larvae are being shed, several examinations over 1 week using a concentration technique may be required to detect the infection (Ash & Orihel, 1987, 2007; Garcia, 2009, 2016). It is important to note that filariform larvae of *S. stercoralis* and hookworms are highly infectious; therefore, testing must be performed using universal precautions.

Objects Resembling Enteric Parasites

A large variety of objects that closely resemble various parasite life cycle stages may be seen in feces and other specimens sent for O&P examination. Careful differentiation of these objects from real parasites is necessary to prevent inappropriate or unnecessary treatment. White blood

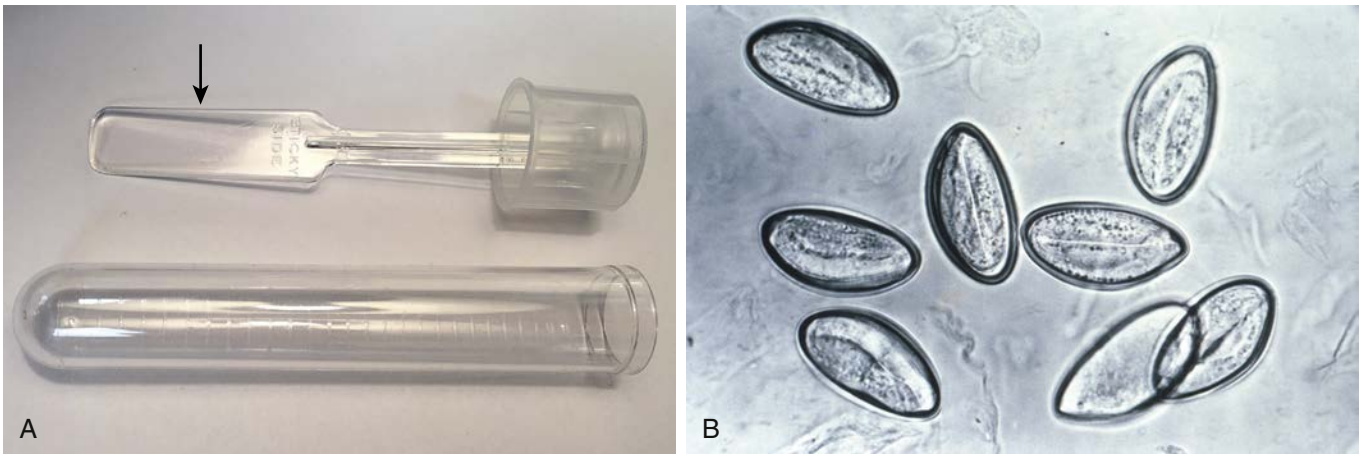


Figure 65.2 **A,** Commercial collection device (Swube) for detection of *Enterobius vermicularis* eggs and adult females from perianal skin. The paddle *(arrow)* has a sticky side that is applied to the perianal skin to collect the specimen. The paddle is then placed into the accompanying tube for safe transport to the laboratory. The clear paddle functions like a glass slide and can be viewed directly under the microscope. **B,** Positive specimens demonstrate *E. vermicularis* eggs and, less commonly, adult females. Eggs measure 50 to 60 μm long by 20 to 30 μm wide and are flattened on one side (unstained, 400×). (**B** courtesy of the Centers for Disease Control and Prevention Public Health Image Library.)

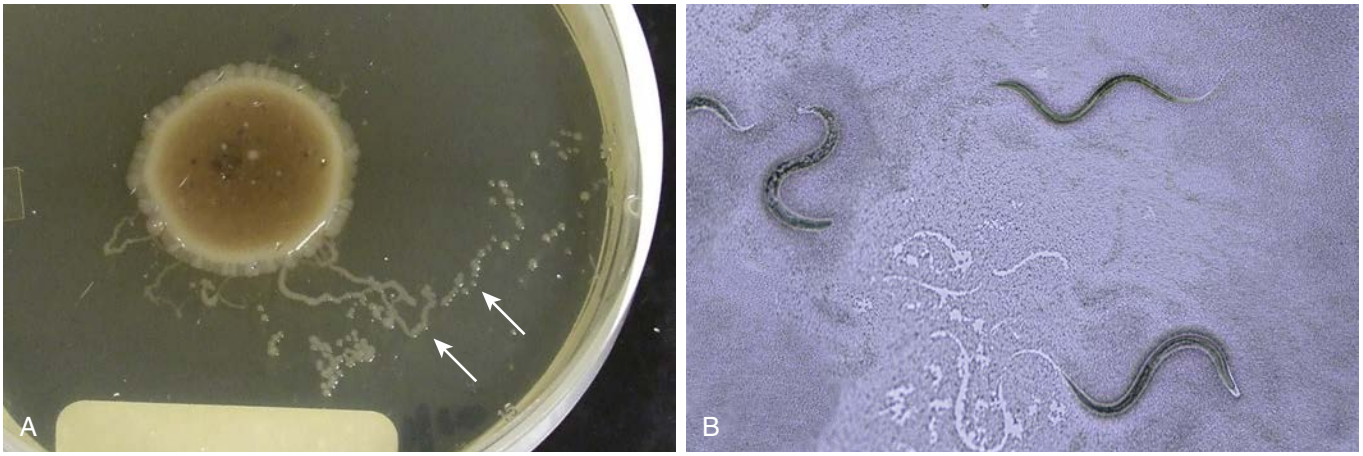


Figure 65.3 **A,** Stool agar culture method showing bacterial tracks *(arrows)* formed by migrating *Strongyloides stercoralis* larvae. **B,** Examination of the plate using a light microscope reveals multiple larvae and detail of the agar tracks (unstained, 100×).

cells, macrophages, and squamous and columnar epithelial cells may resemble amebae; yeasts and starch granules may resemble protozoal cysts or oocysts; pollen and fungal conidia may resemble helminth eggs; plant fibers may resemble nematode larvae; and pieces of vegetables or vegetable skins may resemble adult worms or proglottids. Examples of artifacts and pseudoparasites have been reviewed elsewhere (Ash & Orihel, 2007; Pritt, 2014; Garcia, 2016).

EXAMINATION OF UROGENITAL AND OTHER SPECIMENS (SPUTA, ASPIRATES, BIOPSIES)

Vaginal and urethral discharges, prostatic secretions, or urine may be submitted to the laboratory for detection of *Trichomonas vaginalis*. The most rapid and cost-effective method is the preparation of several wet mounts using a drop of specimen (urine should be centrifuged) diluted with a drop of saline, which is then covered with a coverslip. The slide is examined under the low-power (10×) objective using reduced lighting conditions for motile trophozoites, which display a jerky movement. High-power examination may reveal the beating flagella and the undulating membrane characteristic of the species. Unfortunately, wet mount microscopy is a relatively insensitive method (sensitivity 51%–65%) for detection of *T. vaginalis*; use of NAATs is now recommended for optimal detection (Workowski & Bolan, 2015). Use of culture, fluorescent antibody reagents, or a commercial deoxyribonucleic acid (DNA) probe technique also provides some improvement in sensitivity over wet mount microscopy (Peterman et al., 2006; Schwebke et al., 2011; Schwebke et al., 2018). Demonstration of metronidazole and tinidazole drug resistance requires culture of the organism (Workowski & Bolan, 2015; Clain et al., 2019).

Urine is also an important specimen for detecting the eggs of *Schistosoma haematobium* and, less commonly, microfilariae (Garcia, 2016). Urine can be examined directly for eggs and microfilariae; however, filtration through a membrane filter or examination of urine collected over a 24-hour period may increase the sensitivity of detection (Cheesbrough, 2005). *S. haematobium* eggs are shed sporadically; thus, examination of multiple specimens collected on different days is recommended, with specimens optimally collected between 10 AM and 2 PM (Cheesbrough, 2005).

A number of protozoal and helminthic parasites may be detected in sputum; the appropriate examination technique depends on the suspected organism. Generally, the technique required to detect a parasite from its usual site of infection is applied to sputum and most commonly involves a wet mount. When amebae are suspected, permanent stains should be performed. Acid-fast or specific antibody-based stains are appropriate for detection of *Cryptosporidium* oocysts. Identification techniques for the microsporidia and *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) are described elsewhere.

Examination of aspirates requires the use of stains as appropriate for the implicated organism. In addition to the methods used for sputum, Giemsa staining is often appropriate when examining for protozoa, especially the hemoflagellates. Biopsy material should be submitted for routine histology after imprint smears are prepared for staining with Giemsa or another appropriate permanent stain. Culture for *Leishmania* and trypanosomes also can be performed on tissues and may be important for demonstrating those infections. This testing is usually restricted to specialty reference and public health laboratories, such as the CDC. Skin snips sent for *Onchocerca* or *Mansonella streptocerca* examination should be teased apart in saline and the saline examined after 30 to 60 minutes for microfilariae. Muscle biopsy specimens for *Trichinella* spp. larvae may be examined by compressing the fresh specimen between two glass slides or by submitting it for routine histology. Likewise, rectal or bladder biopsies may be examined for schistosome eggs.

PARASITE CULTURE TECHNIQUES

Culture methods have been described for a wide variety of protozoan parasites, but few clinical laboratories undertake the task because of infrequent requests and lack of familiarity with methods. When culture requests are made, they are usually for *T. vaginalis*, *Leishmania* spp., *Trypanosoma cruzi*, *E. histolytica*, *Acanthamoeba* spp., or *Naegleria fowleri*. Methods are reviewed elsewhere (Ash & Orihel, 1987; Garcia, 2009, 2010, 2016; Garcia et al., 2018). The CDC may agree to provide this testing following consultation.

IMMUNODIAGNOSTIC METHODS

Several immunodiagnostic methods are available to identify the parasitic antigen or the antibody that is produced in response to the parasitic infection. Some signals are amplified, and others are direct detection methods. In general, laboratory methods employed are enzyme immunoassay (EIA), indirect immunofluorescence assay (IFA), direct fluorescence antibody assay (DFA), Western blot, radioimmunoassay, and immunodiffusion, among others.

Antigen Detection

Antigen detection methods are commercially available for several parasitic diseases, including amebiasis, cryptosporidiosis, giardiasis, malaria, and trichomoniasis (Table 65.4). These methods may be useful for initial testing or in instances in which traditional tests are negative, yet a high index of clinical suspicion remains. These tests offer the advantage of detecting current infection and can often be performed by someone other than an experienced morphologist (Garcia et al., 2018; Shimizu & Garcia, 2018).

Antigen detection in stool samples is usually performed using fecal immunoassays. A number of published studies have suggested that these assays have good or superior sensitivity and specificity when compared with routine ova and parasite examination (Cama & Mathison, 2015; Garcia et al., 2018). These immunoassays are easy to use and rapid, permit batch processing, and do not require experienced microscopists. Given the current shortage of medical technologists and individuals with specialized training in parasitology, use of immunoassays appears to be an attractive alternative. However, laboratories that use rapid cartridge-based immunoassays should be aware of potential problems with false-positive results and should closely monitor test performance. Currently, fecal immunoassays are marketed for *G. duodenalis*, *C. parvum/C. hominis*, the *E. histolytica/E. dispar* group, and *E. histolytica*. Antigen detection tests using blood or serum are also available for *Plasmodium* spp. and *W. bancrofti*. A latex agglutination test for *T. vaginalis* antigen detection in vaginal swabs has also been introduced. Immunoassays are usually available in three formats: EIA, DFA, and lateral flow (immunochromatography) cartridges. Fresh or preserved stool samples are appropriate for most antigen detection kits (Garcia et al., 2018). Although each kit has unique operating characteristics, most are generally comparable in performance (Garcia, 2016; Garcia et al., 2018).

TABLE 65.4
Select Antigen-Based Detection Assays for Parasites

Target Organism(s)	Test System	Manufacturer/Distributor	Format	FDA Approval/Clearance*
Cryptosporidium spp.	Xpect Cryptosporidium	Remel (Thermo Scientific)	LFA	Yes
	Crypto Cel	CeLLabs	DFA	Yes
	ProSpecT Cryptosporidium	Remel (Thermo Scientific)	EIA plate	Yes
	Cryptosporidium II	Tech-lab	EIA plate	Yes
	RIDASCREEN Cryptosporidium	R-Biofarm	EIA	No
	PARA-TEC Cryptosporidium	Medical Chemical Corporation	EIA	No
	UNI-GOLD Cryptosporidium	Trinity Biotech	LFA	Yes
	Crypto-Strip C-1005 (CRYPTO UNISTRIP, CRYPTO-CIT)	Coris BioConcept	LFA	No
	CRYPTO (card and blister formats)	CerTest Biotec	LFA	No
	Stick Crypto	Operon	LFA	No
Giardia duodenalis	Xpect GIARDIA	Remel (Thermo Scientific)	LFA	Yes
	Uni-Gold Giardia	Trinity Biotech	LFA	No
	Stick Giardia	Operon	LFA	No
	Giardia (dipstick and cassette)	Coris BioConcept	LFA	No
	Giardia lamblia (Giardia)	CerTest Biotec	LFA	No
	ProSpecT Giardia EZ	Remel (Thermo Scientific)	EIA plate	Yes
	Giardia lamblia ANTIGEN DETECTION MICROWELL ELISA	IVD Research	EIA plate	Yes
	Giardia Cel	CeLLabs	DFA	Yes
	ProSpecT Giardia	Remel (Thermo Scientific)	EIA plate	Yes
	RIDASCREEN Giardia	R-Biopharm	EIA plate	No
	PARA-TEC Giardia	Medical Chemical Corporation	EIA plate	No
	GIARDIA II	Tech-lab	EIA plate	Yes
	Giardia CELISA	CeLLabs	EIA plate	Yes
	Giardia lamblia II	Tech-lab	EIA plate	Yes
Entamoeba histolytica	E. histolytica QUIKCHEK	Tech-lab	LFA	Yes
	Entamoeba CELISA PATH	CeLLabs	EIA plate	Yes
	ProSpecT Entamoeba histolytica	Remel (Thermo Scientific)	EIA plate	Yes
	E. histolytica II	Tech-lab	EIA plate	Yes
Cryptosporidium/Giardia	Xpect Giardia/Cryptosporidium	Remel (Thermo Scientific)	LFA	Yes
	ImmunoCard STAT! Cryptospo-ridium/Giardia	Meridian Bioscience	LFA	No
	RIDA Quick Cryptosporidium/Giar-dia Combi (dipstick or cassette)	R-Biopharm	LFA	No
	CRYPTO-GIARDIA	CerTest Biotech	LFA	No
	MERIFLUOR Cryptosporidium/ Giardia	Meridian Bioscience	DFA	Yes
	Crypto Giardia DFA	IVD Research Inc.	DFA	Yes
	Crypto/Giardia Cel	CeLLabs	DFA	Yes
	PARA-TECT Cryptosporidium/ Giardia	Medical Chemical Corp.	DFA	No
	ColorPAC Giardia/Cryptosporidium	Becton Dickinson (BD)	LFA	Yes
	Giardia/Cryptosporidium CHEK	Tech-lab	EIA plate	Yes
	Giardia/Cryptosporidium QUI-KCHEK	Tech-lab	LFA	Yes
	ProSpecT Giardia/Cryptosporidium	Remel (Thermo Scientific)	EIA plate	Yes
Giardia duodenalis, *Cryptosporidium* spp., and *E. histolytica/dispar* combination	Alere Triage Parasite Panel	Alere	LFA	Yes
	TRI-COMBO Parasite Screen	Tech-lab	EIA plate	Yes
	RIDA Quick Cryptosporidium/Giar-dia/Entamoeba Combi (dipstick or cassette)	R-Biopharm	LFA	No
	CRYPTO-GIARDIA-ENTAMOEBA	CerTest Biotec	LFA	No
Plasmodium spp.*	BinaxNOW Malaria	Alere	LFA	Yes
	OptiMAL	BIO-RAD	LFA	No
	First Response Malaria Ag	Premier Medical Corp.	LFA	No
	CareStart Malaria COMBO	Apacor	LFA	No
	SD BIOLINE Malaria Ag	Alere	LFA	No
Wuchereria bancrofti	BinaxNOW Filariasis	Alere	LFA	No
	Filariasis Ab CELISA	CeLLabs	EIA plate	No

TABLE 65.4 —cont'd

Target Organism(s)	Test System	Manufacturer/Distributor	Format	FDA Approval/Clearance*
Trichomonas vaginalis	Light Diagnostic T. vaginalis	Nippon Chemicon	DFA	No
	OSOM Trichomonas Rapid Test	Sekisui Diagnostics	LFA	Yes
	XenoStrip-Tv	Xenotope Diagnostics	LFA	No

DFA, Direct fluorescence antibody assay; *Dipstick,* dipstick enzyme immunoassay; *EIA,* enzyme immunoassay; *LFA,* lateral flow assay.
*Many antigen detection tests are commercially available for *Plasmodium* spp. The tests listed here scored highly for detection of *P. falciparum* by the World Health Organization and the Foundation for Innovative New Diagnostics (FIND) product testing (FIND, 2012). Tests listed detect both *P. falciparum* and Pan-*Plasmodium* antigens.

Rapid antigen detection tests (RDTs) developed for malaria may detect histidine-rich protein II (HRP-II), parasite lactate dehydrogenase (pLDH), parasite aldolase, or a combination of these antigens in peripheral blood. HRP-II tests are specific for *Plasmodium falciparum*, and pLDH and aldolase tests detect all four human *Plasmodium* spp. These assays have highly variable performance characteristics, but in general are adequate for detecting moderate to heavy infections with *Plasmodium falciparum*. They are often significantly less sensitive for detecting lower levels of *P. falciparum* infection and infections with other *Plasmodium* spp. At the time of this writing, only the BinaxNow (Abbott Diagnostics, Chicago, IL) is cleared by the U.S. Food and Drug Administration (FDA) for clinical diagnosis of malaria in the United States (Mathison & Pritt, 2017).

Trichomonas vaginalis antigens from vaginal samples may also be detected using rapid antigen tests such as the OSOM Trichomonas Rapid Test (Sekisui Diagnostics, Burlington, MA), which is an FDA-cleared, Clinical Laboratory Improvement Amendments (CLIA)–waived test for detecting *T. vaginalis* antigen in vaginal swabs. These tests can be used for rapid detection of *T. vaginalis* infection in the clinical setting and may replace wet mount examinations, which generally have lower sensitivities (75%–96%) compared with NAATs (Association of Public Health Laboratories [APHL], 2016).

Most EIAs are available in microwell format (Shimizu & Garcia, 2019). Antigens from frozen, fresh, or 10% formalin–preserved stool samples are suitable for testing by this method. Concentrated or PVA samples are not suitable for testing with EIA kits. Parasite antigen is captured by immobilized antibodies coated on microwells and is detected by an enzyme-conjugated secondary antibody that is capable of producing a colored reaction following the addition of substrate. Although the colored wells can be read visually or with the use of a spectrometer, the latter seems to be the preferred option because of occasional ambiguous results obtained with some kits (Garcia et al., 2018). In general, EIA tests have good sensitivities and specificities. Garcia and colleagues evaluated nine immunoassay kits for detection of *G. duodenalis* and *Cryptosporidium* spp. in comparison with a reference DFA test that visualizes the parasite directly in the sample. Investigators found that all kits had high sensitivities, ranging from 94% to 99%, and 100% specificities (Garcia & Shimizu, 1997, 2000). This is in contrast to first-generation EIAs, which had been previously reported to produce false-positive results, resulting in their recall (Doing et al., 1999). Hence, a strong quality control (QC) pro and participation in proficiency test pros are required to ensure high-quality test results. Local epidemiology of the parasitic infection can help to determine whether additional confirmatory testing is required; consultation with local public health authorities may prove useful in characterizing which infections are being seen locally. Additionally, for some diseases such as giardiasis, examination of two specimens by EIA or microscopy may be necessary to achieve diagnostic sensitivity greater than 90% (Hanson & Cartwright, 2001).

Lateral flow cartridges are a popular format of immunoassay because of their ease of use and the minimal performance time required. These kits can be stored conveniently at room temperature and may be used in single or batch processing. The parasite antigen in the sample migrates through the membrane and binds to specific capture antibodies. Use of a secondary reagent results in development of a colored reaction. These kits also have an internal control to ensure that the colloidal dye conjugates used in the assay are intact and that proper capillary flow has occurred. To ensure complete migration of the specimen, only the supernatant of a well-mixed stool sample is used, and some samples may be diluted to a liquid state before testing. Any color visible at the reagent test zone (usually a band) is interpreted as positive. Some studies have demonstrated that cartridge assays are somewhat less sensitive than a microwell EIA plate assay (Pillai & Kain, 1999; Johnston et al., 2003). When lack of sensitivity is a concern, it may be necessary to perform alternative O&P or NAAT studies if the patient's symptoms persist.

DFA testing allows direct visualization of the parasites in stool specimen using antibodies conjugated to fluorescent dyes. These assays are easy to perform and to interpret, permitting rapid screening of slides when compared with some of the traditional stains used in parasitology (Garcia et al., 2018). A fluorescence microscope is necessary for this procedure, which is a limiting factor in some laboratories. Currently, kits are available for detection of cysts of *G. duodenalis* and oocysts of *Cryptosporidium* spp. Fixed stool specimens may be used for this procedure (10% formalin, SAF, or one of the mercury- or formalin-free products) (Garcia et al., 2018). Although fresh stool samples can be tested directly, the sensitivity of the assay can be improved by performing the test on centrifuged stool (500 *g* for 10 minutes). Occasionally, fluorescing bacteria and yeasts may be seen, but these are readily distinguished from *Giardia* and *Cryptosporidium* on the basis of their size and shape. The edges of the wells should be carefully examined to avoid missing the rare parasite in light infections. Given the relatively recent recognition of additional *Cryptosporidium* spp. that may infect humans, commercial assays currently available may not be adequate for detection of all infections.

Antibody Detection

Tests that are available from public health, hospital, or commercial laboratories to detect immunologic reactivity to parasitic diseases are summarized in Table 65.5. Historically, serologic procedures for parasitic diseases have been plagued by low sensitivity and specificity, primarily owing to the complex antigenic nature of parasites and the possibilities for cross-reactions from related species. However, newer test methods combined with the use of more highly defined antigenic components has provided more accurate results with greater predictive values. Many of the newer tests use the EIA or immunoblot (Western blot) format, although IFA, indirect hemagglutination (IHA), and complement fixation (CF) are still in use (Wilkins & Nutman, 2015).

In general, serologic diagnosis of parasitic infection is used as an adjunct to the usual diagnostic modalities or in special situations in which identification of the parasite itself or its antigen or nucleic acid from host tissue or excreta is not possible (Wilkins & Nutman, 2015). For example, parasitic infections such as toxoplasmosis and toxocariasis reside in deep tissues and cannot be readily diagnosed by morphologic means. Others such as cysticercosis and echinococcosis develop in organs, where invasive studies that may be required are not recommended in the initial patient evaluation. Additional conditions—such as filariasis, schistosomiasis, and strongyloidiasis—may remain subclinical because of light infections or because the clinical evaluation occurred during the prepatent period (interval from infection to demonstration of symptoms or recovery of organism). In these settings, serologic testing may be invaluable for making a presumptive diagnosis in correlation with the accompanying clinical and radiographic features. Other circumstances in which serologic evaluation may prove useful include diagnosis of extraintestinal amebiasis (e.g., amebic liver abscess), trichinellosis, and chronic stages of trypanosomiasis. Lastly, serologic studies serve as a powerful tool in enhancing our understanding of the epidemiology of diseases such as schistosomiasis, toxoplasmosis, amebiasis, Chagas disease, malaria, and babesiosis, and for screening blood donors for select infections (e.g., malaria, Chagas disease) (Wilkins & Nutman, 2015).

Interpretation of serologic testing may be challenging. Detection of antibodies, especially immunoglobulin (Ig) G, provides evidence of infection but may not be able to differentiate active from past exposure. High antibody levels are useful for diagnostic purposes if they occur in a patient with no previous exposure to the parasite and no recent history of travel to an endemic area. Unfortunately, positive antibody levels in persons living in endemic areas often do not help in the clinical diagnosis. In some parasitic diseases, levels of antibodies may decline slowly following successful therapy or self-cure and thus may be useful for monitoring response to treatment (Wilkins & Nutman, 2015).

Serologic tests for parasitic diseases generally evaluate IgG levels with the exception of toxoplasmosis and babesiosis, in which IgM- and IgA-specific antibodies may be helpful for determining the age of infection (Wilson & Nutman, 2015). Unfortunately, IgM and IgA may persist for as long as 2 years after the primary infection, thus limiting their utility for differentiating acute from past infection. When *Toxoplasma gondii* IgG

TABLE 65.5
Examples of Serologic Assays for Parasites Available from Reference Laboratories

Disease	Organism	Specimen Type	Assay
Amebiasis	*Entamoeba histolytica*	Serum	EIA, ID, IHA
Baylisascariasis	*Baylisascaris procyonis*	Serum, CSF	IB
Babesiosis	*Babesia microti*, *Babesia* sp. WA1	Serum	IFA
Chagas	*Trypanosoma cruzi*	Serum	IFA, EIA, CF, IB
Cysticercosis	*Taenia solium*	Serum, CSF	EIA, IB
Echinococcosis	*Echinococcus granulosus*	Serum	EIA, IB, IHA, IFA
Fascioliasis	*Fasciola hepatica*	Serum	IB
Filariasis	*Wuchereria bancrofti*	Serum	EIA
Leishmaniasis	*Leishmania braziliensis*, *Leishmania donovani*, *Leishmania tropica*	Serum	IFA, EIA, CF
Malaria	*Plasmodium* spp.	Serum	IFA
Paragonimiasis	*Paragonimus westermani*	Serum	EIA, IB
Schistosomiasis	*Schistosoma* spp.	Serum	EIA, IB
Strongyloidiasis	*Strongyloides stercoralis*	Serum	EIA
Toxoplasmosis	*Toxoplasma gondii*	CSF, serum	IFA, EIA
Trichinellosis	*Trichinella spiralis*	Serum	EIA, BF

BF, Bentonite flocculation; *CF,* complement fixation; *CSF,* cerebrospinal fluid; *EIA,* enzyme immunoassay; *IB,* immunoblot; *ID,* immunodiffusion; *IFA,* indirect immunofluorescence assay; *IHA,* indirect hemagglutination.

antibodies are detected, avidity testing may also be useful to distinguish recent from past infection, particularly during pregnancy (Wilson & Nutman, 2015; Robert-Gangneux & Dardé, 2012; McAuley & Singh, 2019). This testing is based on the principle that the initial host response results in the production of low-avidity antibodies. Over time, the antibodies gain higher avidity as the host immune response is enhanced.

Because serologic tests for most parasitic diseases are requested infrequently, specimens generally are submitted to public (CDC) or private reference laboratories. Some of the more commonly requested tests are available as commercial kits. However, many of these assays are developed in-house and, hence, lack correlation with universal standards. Interpretive criteria are established by reagent manufacturers or by the center performing the test; these criteria often vary from institution to institution. Individuals requesting such tests should inquire about the performance characteristics, including sensitivity and specificity, and should be aware that cross-reactions may occur. For example, antibody tests for Chagas disease are known to cross-react with antibodies produced in response to *Leishmania* infections. However, reactivity to homologous antigen is greater, and this test is useful in diagnosing chronic stages of the disease when parasitemia is generally low. Usually, serology for chronic Chagas disease correlates well with molecular diagnostic methods (Afonso et al., 2012). Helminthic parasites are well known to cross-react in serologic assays that use crude antigen preparations because of phylogenetic, hence antigenic, similarities.

Several factors that may influence the test performance of serologic assays include disease manifestation, test format, reagents used, and parasite viability, to name a few. The sensitivity of the test is increased in patients with invasive amebiasis but may be weak in intestinal amebiasis with minimal tissue invasion and absent for asymptomatic carriers. The type of serologic assay format may also determine the sensitivity, as in the diagnosis of toxoplasmosis (Wilkins & Nutman, 2015). The double-sandwich IgM enzyme-linked immunosorbent assay (ELISA) is known to be more sensitive and specific than IgM immunofluorescence for detecting recently acquired and congenital toxoplasmosis. IHA has been the primary test for serodiagnosis of amebiasis. The sensitivity of the assay is also dependent on the type or stage of parasite antigen used. For example, the sensitivity of cutaneous leishmaniasis can be improved by using amastigote antigens in place of promastigote antigens in the IFA test. Finally, serologic assays are also affected by parasite viability; hydatid cysts occurring in the lung and dead or calcified cysts are less frequently detected than active cysts in the liver. This also holds true for neurocysticercosis (larval infection with the cestode *Taenia solium*), in which sensitivity is low when only a single parenchymal cyst or calcified lesions are present (White et al., 2018).

MOLECULAR DIAGNOSTIC METHODS

Diagnostic methods using DNA and ribonucleic acid (RNA) amplification and nucleic acid probe techniques have been described for most of the common parasitic diseases and, in general, offer high levels of sensitivity and specificity. For more complete details on this topic, the reader is referred to more recent publications (Wilkins & Nutman, 2015; Pritt, 2015). Molecular methods offer some unique advantages, such as high sensitivity and specificity, and the ability to detect and differentiate species variants—all independent of the patient's underlying immune status, which is a potentially limiting feature of serologic assays. On the other hand, molecular amplification techniques—particularly those using "open" formats in which amplified nucleic acid is manipulated, are prone to cross-contamination if proper processing precautions are not strictly enforced.

The availability of molecular tests has been greatly enhanced by the introduction of multiple commercial assays, some of which are FDA cleared for in vitro diagnostic use (Wilkins & Nutman, 2015; Pritt, 2015). Among the FDA-cleared tests are assays for *T. vaginalis*, *G. duodenalis*, *Cryptosporidium* spp., *E. histolytica*, and *Cyclospora cayetanensis*. Laboratory-developed NAATs have also been developed for parasites such as *Plasmodium* spp., *Babesia* spp., *Leishmania* spp., *T. gondii*, and *Trypanosoma* spp. and are available through specialized reference and public health laboratories (Table 65.6). Most assays today are available in real-time format, wherein the kinetics of the nucleic acid amplification reaction is recorded and analyzed by computer algorithms to allow detection of amplicons (Wilkins & Nutman, 2015). The introduction of this technology has allowed for rapid detection and has lessened the risk for amplicon cross-contamination due to the closed nature of the steps involved in postamplification analysis. Isothermal methods—such as strand displacement amplification, transcription-mediated amplification, and loop-mediated isothermal amplification (LAMP) have also been described for some parasites and allow for rapid and highly sensitive detection of parasite nucleic acid. Because the innate nature of molecular methods is genotypic, polymerase chain reaction (PCR) assays have the ability to accurately detect to the species level depending on the gene being targeted, and may also be used for strain typing and outbreak investigation (Wilkins & Nutman, 2015). Molecular methods may also allow for detection of mutations associated with drug resistance (Wilkins & Nutman, 2015; Pritt, 2015; Clain et al., 2019).

QUALITY ASSURANCE, QUALITY IMPROVEMENT, AND SAFETY

A quality assurance program for the parasitology section of the laboratory is similar to that for the other laboratory sections. It covers all essential aspects of the operation, including, among others, a well-written and complete procedure manual that is reviewed annually, guidelines for maintaining all specimen and test result records, a complete QC program with appropriate technical supervision and review, and participation in an approved proficiency testing program. Laboratories also need to focus on customer satisfaction using a variety of available measures and should participate in the team approach to identifying problems and generating solutions as part of a continuous quality improvement process (Garcia, 2016).

TABLE 65.6
Examples of Nucleic Acid Amplification Assays for Parasites

Parasite	Common Target(s)	Specimen Type	FDA-Approved/Cleared Assay Available (Assay, Manufacturer)
Leishmania spp.	rDNA, kinetoplastid DNA, ITS1 and ITS2 genes	Whole blood, skin scrapings, tissue	Yes* (SMART Leish PCR, U.S. Army)
Plasmodium spp.	rDNA	Whole blood	No
Toxoplasma gondii	RE and B1 genes	Amniotic fluid, blood, cerebrospinal fluid, tissue, whole blood, ocular fluid	No
Entamoeba histolytica	rDNA	Stool	Yes† (FilmArray GI panel, BioMerieux; xTAG Gastrointestinal Pathogen Panel, Luminex Corporation; BD MAX Enteric Parasite Panel, BD)
Giardia duodenalis	rDNA, β-Giardia gene	Stool	Yes† (FilmArray GI panel, BioMeriux; xTAG Gastrointestinal Pathogen Panel, Luminex Corporation; BD MAX Enteric Parasite Panel, BD)
Cryptosporidium spp.	rDNA	Stool	Yes† (FilmArray GI panel, BioMerieux; xTAG Gastrointestinal Pathogen Panel, Luminex Corporation; BD MAX Enteric Parasite Panel, BD)
Cyclospora cayetanensis	rDNA	Stool	Yes† (FilmArray GI panel, BioFire Diagnostics)
Trichomonas vaginalis	rDNA, β-Tubulin gene	Vaginal, cervical and urethral (male) samples, urine, semen	Yes (Aptima Trichomonas vaginalis assay, Hologic; Xpert TV,‡ Cepheid; Solana Trichomonas assay, Quidel; BD Max CT/GC/TV, BD)

FDA, U.S. Food and Drug Administration; *PCR,* polymerase chain reaction; *rDNA,* ribosomal deoxyribonucleic acid.
*Restricted to U.S. Department of Defense use.
†Component of multiplex assays for gastrointestinal pathogens.
‡Only FDA-approved assay for testing both males and females.

The performance of individuals responsible for the parasitology section should be monitored periodically with both internal and external unknown specimens, and competency assessments should be up-to-date, especially for those laboratories that encounter positive specimens infrequently. A variety of reference materials should be readily available for use at the laboratory bench, including positive slides and fecal specimens, printed atlases, and slide atlases.

Unpreserved specimens for parasitologic examination should be considered potentially infectious. Thus, all blood and body fluids should be handled according to Standard Precautions as defined by the Final Rule on Blood-borne Pathogens by the Occupational Safety and Health Administration, as published in the *Federal Register*. In addition to blood-borne viral pathogens, malarial parasites and hemoflagellates may remain infective. A variety of parasites may remain infective in fresh stool specimens, including cysts of enteric protozoa; eggs of *Taenia solium*, *E. vermicularis*, and *H. nana*; and filariform larvae of *S. stercoralis*. *Trichuris trichiura*, *Ascaris lumbricoides*, and hookworm eggs may remain infective in older specimens, and *Ascaris* eggs can survive and embryonate while in 5% formalin. Fecal specimens also may contain pathogens such as *Salmonella*, *Shigella*, or viruses. Strict observance of proper specimen handling techniques and disposal is essential. Personal attention to hand washing is also necessary.

BLOOD AND TISSUE PROTOZOA

PLASMODIUM SPP. (MALARIA)

Malaria (from the Italian *mal' aria*, meaning "bad air") is an acute and sometimes chronic infection of the bloodstream characterized clinically by fever, anemia, and splenomegaly, and is caused by apicomplexan parasites of the genus *Plasmodium*. The defining clinical features of a malarial attack or paroxysm consist of, in order, shaking chills, fever (up to 40°C or higher), and generalized diaphoresis, followed by resolution of fever. The paroxysm occurs over 6 to 10 hours and is initiated by the synchronous rupture of erythrocytes with the release of new infectious blood stage forms known as *merozoites* (Fig. 65.4). The disease generally occurs throughout the tropics and subtropics and is spread exclusively by female anopheline mosquitoes. The four main species of *Plasmodium* causing human malaria are *P. vivax*, *P. falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. *P. falciparum* infection occurs principally in tropical areas worldwide, whereas *P. vivax* infections occur in both tropical and temperate zones, including the east horn of Africa, Central Asia and the Indian Subcontinent, Southeast Asia, and Latin America. *P. malariae* also occurs worldwide but to a much lesser extent than *P. falciparum* or *P. vivax*. *Plasmodium ovale* occurs primarily in western Africa and Southeast Asia; interestingly, *P. ovale* is the only species to date not to have been introduced to the Americas. Recently, human infection with *P. knowlesi*, a malarial parasite of Old World monkeys, has been described in several regions of Southeast Asia. These infections are potentially life-threatening but are difficult to distinguish from *P. malariae* microscopically, leading to misidentification. Use of PCR may be required to make the correct differentiation (Cox-Singh et al., 2008).

Because infection with *falciparum* malaria is potentially life-threatening, its presence must be considered in the differential diagnosis of unexplained fever, and history of travel in endemic geographic areas should always be sought. In an era of increasing world travel, the risk for acquiring malaria is not insignificant, and the rapid spread of drug-resistant strains poses particular problems when appropriate prophylaxis or therapy is considered.

Laboratory evaluation of patients suspected of having malaria continues to rely on timely examination of thick and thin blood films to demonstrate the intraerythrocytic parasites. Although they are straightforward in their approach, performance of these techniques may be problematic. Reliable identification of organisms requires continuous training to maintain expertise; therefore, those laboratories that rarely see positive specimens may choose to refer specimens to reference laboratories provided that processing and reporting are timely.

NAATs for detection of parasite-specific DNA (Mathison & Pritt, 2017) provide enhanced sensitivity and specificity but generally are not appropriate or available for smaller laboratories. They are also not typically performed on a STAT basis, as is necessary for detection of acute disease.

Within the past decade, antigen detection methods have become widely available for use in endemic and nonendemic settings (see Laboratory Methods section earlier in this chapter). They are now used in many moderate- to large-sized laboratories in nonendemic settings for rapid malaria diagnosis in resource-limited settings, particularly when skilled and experienced microscopists are not available (e.g., night shift). They are also an integral part of the WHO's malaria control efforts; an estimated 276 million malaria RDTs were sold worldwide in 2017, with an additional 245 million RDTs distributed at no cost by the National Malaria Programme (WHO, 2018). The WHO estimates that 75% of malaria tests conducted in malaria-endemic regions in sub-Saharan Africa were via RDTs (WHO, 2018). Most commercially available tests utilize a lateral flow format and detect *Plasmodium*-specific antigens such as lactate dehydrogenase, aldolase, and/or *P. falciparum* HRP-II. While they generally provide a high degree of sensitivity and specificity for the diagnosis of falciparum malaria, most suffer from inadequate sensitivity for detection of lower parasite loads and non–*P. falciparum* infections. The sensitivity, specificity, and invalid rates vary widely among the malaria RDTs. The WHO has performed 7 rounds of product testing on commercially available RDTs, the results of which are freely available online (WHO, 2017). It is generally recommended that antigen-based assay testing be followed by confirmatory conventional thick and thin film examination (Wilson, 2012; Mathison & Pritt, 2017).

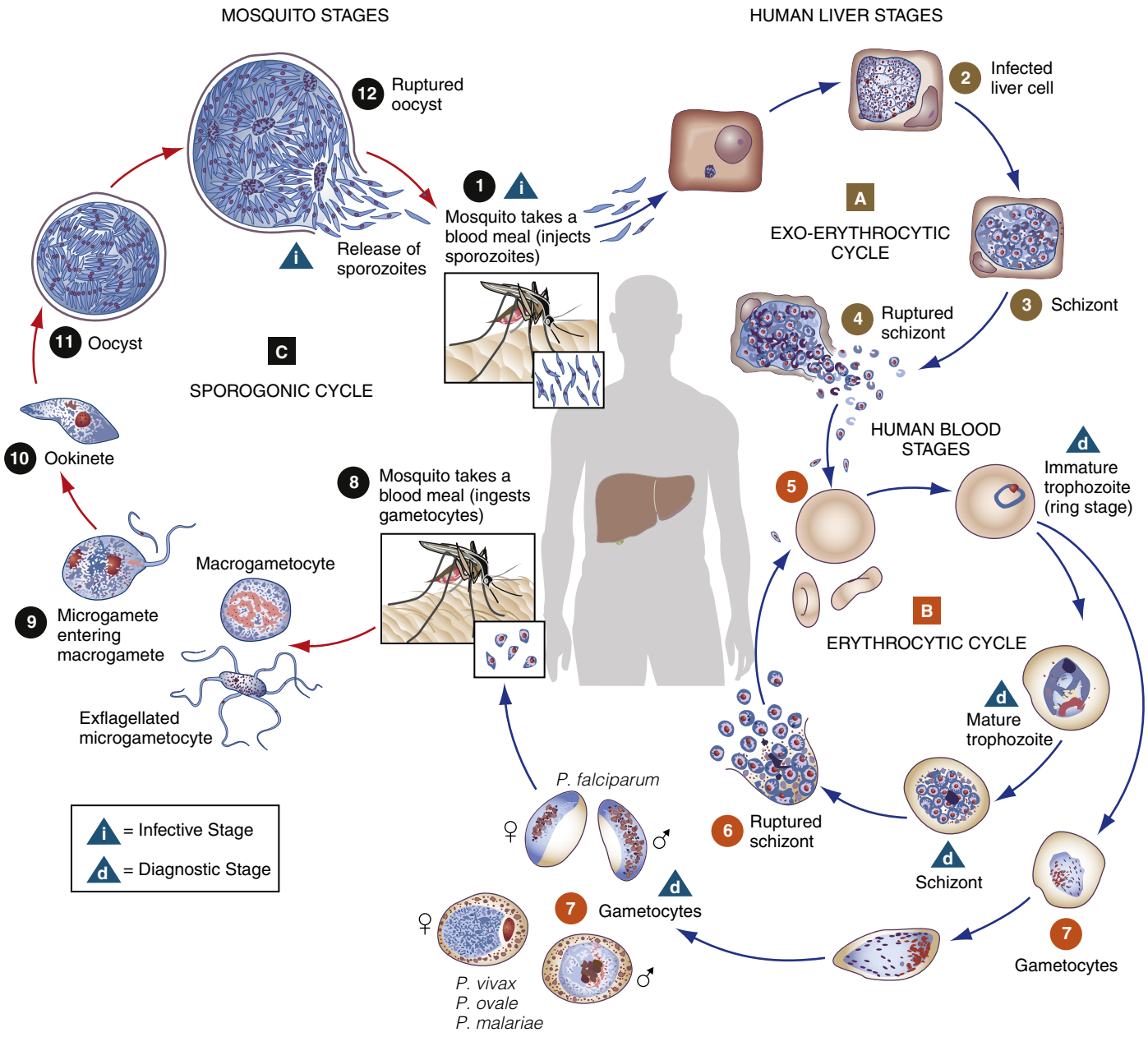


Figure 65.4 Life cycle of *Plasmodium* spp. Infection occurs when an infected female anopheline mosquito injects infectious sporozoites while taking a blood meal (1). Sporozoites are carried to the liver via the bloodstream, where they infect a hepatocyte (2) and divide asexually to form a hepatic schizont (3). The schizont ruptures (4), releasing infectious merozoites into the bloodstream that infect erythrocytes (5) and initiate the erythrocytic cycle. The early stage of the parasite is a "ring-form" trophozoite. As the trophozoite matures, it takes one of two pathways: it can become an erythrocytic schizont (6) that perpetuates the erythrocytic cycle or it can become a male or female gametocyte (microgametocyte or microgametocyte, respectively), (7). The gametocyte is a dead-end stage in the human host and is taken up by a mosquito when it takes a blood meal (8) to initiate the sexual cycle. In the mosquito host, microgametocytes (males) form microgametes and exflagellate, releasing the individual microgametes. A microgamete will fertilize a macrogamete (female) (9), resulting in the formation of an ookinete (10). The ookinete matures and becomes an oocyst in which proliferation of sporozoites occurs (11). The mature oocyte ruptures, releasing infectious sporozoites (12) which migrate to the salivary glands of the mosquito. The cycle continues when a female mosquito takes her next blood meal and injects sporozoites into the human host. (Courtesy of DPDx, Centers for Disease Control and Prevention, Atlanta.)

Life Cycle

Plasmodium spp. have a complex life cycle involving a mosquito definitive host and a vertebrate intermediate host (see Fig. 65.4). Human infection is initiated when a female *Anopheles* mosquito takes a blood meal and injects infectious sporozoites into the bloodstream. Sporozoites are carried to the liver and invade hepatic parenchymal cells, initiating a proliferative phase known as *extraerythrocytic sporogony*. Eventually, these hepatic schizonts rupture, releasing merozoites into the bloodstream, which infect erythrocytes. Developing parasites are known as *trophozoites*. Early trophozoites are usually characterized by a thin or thick vacuolated cytoplasm and one or two chromatin dots and are referred to as *rings* or *ring-form trophozoites*. Hemozoin pigment, a breakdown product of hemoglobin, is characteristic of erythrocytes containing mature stages of malarial parasites but is not usually evident in ring forms. As the trophozoites mature, they take one of two pathways.

In one pathway, trophozoites become *erythrocytic schizonts* that produce merozoites and perpetuate the erythrocytic cycle. The erythrocytic cycle takes approximately 48 hours (tertian periodicity) for *P. falciparum*, *P. ovale*, and *P. vivax* infections, and 72 hours (quartan periodicity) for *P. malariae* infection. Clinical symptoms of fever spikes are ties to the rupturing of erythrocytic schizonts. In the second pathway, trophozoites become macro- (female) or microgametocytes (male). Gametocytes are a dead-end stage in the human host; their sole purpose is to be picked up by a mosquito host to initiate the sexual cycle. In the mosquito host, microgametocytes exflagellate and the released microgametes fertilize a macrogamete, resulting in the formation of a motile *ookinete*. Ookinetes migrate outside the gut wall of the mosquito and become an oocyst. Within the oocyst, sporogony results in the formation of infectious sporozoites. The mature oocyst ruptures into the body cavity, releasing sporozoites, which then migrate through the tissues to the salivary glands, from which they are injected into the vertebrate host as the mosquito feeds. The time required for development in the mosquito ranges from 8 to 21 days.

Plasmodium vivax and *P. ovale* are unique in that true disease relapses may occur weeks to months following subsidence of previous attacks. This

occurs as a result of renewed extraerythrocytic and, eventually, erythrocytic schizogony from latent hepatic parasite forms known as *hypnozoites*. Recurrences of disease due to *P. falciparum* or *P. malariae*, called *recrudescences*, arise from increased numbers of persisting blood stage forms to clinically detectable levels and not from persisting liver stage forms. Liver cells are infected only by sporozoites from the mosquito; thus, transfusion-acquired *P. vivax* or *P. ovale* infection does not relapse (Pritt, 2019).

Epidemiology

Endemic transmission of malaria requires a reservoir of infection, an appropriate mosquito vector, and a susceptible host. Control of malaria is directed at elimination of mosquito hosts, treatment of active cases, and prophylaxis of susceptible persons. However, the emergence of mosquitoes resistant to insecticides, the development of resistance to prophylaxis and therapy by *P. falciparum* and *P. vivax* (WHO, 2018) and lack of adequate funding have made control difficult in endemic areas. Fortunately, renewed global efforts have dramatically decreased the morbidity and mortality of malaria worldwide, although further efforts are needed to achieve full control and elimination (WHO, 2018).

A number of hereditary erythrocyte genotypes offer some degree of protection against severe *P. falciparum* malaria, including sickle cell trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hereditary ovalocytosis, and thalassemia (Mohandas & An, 2012; Uyoga et al., 2019). Alterations of erythrocyte cell membrane surface proteins may interfere with parasite invasion and confer a protective effect against malaria infection and severe disease. Individuals who lack the Duffy blood group antigen, for example, have a decreased likelihood of *P. vivax* infection since this is the primary protein used by *P. vivax* to bind and invade erythrocytes (Zimmerman, 2013). Alternate binding pathways have recently been identified for *P. vivax*, providing an explanation for the numerous reports of *P. vivax* malaria in Duffy blood group–negative individuals (Ntumngia et al., 2016).

Transfusion-acquired malaria may occur when blood donors have subclinical malaria and may prove fatal for the recipient. Similarly, congenital malaria may occur in infants born to mothers from endemic areas. The infant acquires the infection at birth as a result of rupture of placental blood vessels leading to maternal-fetal transfusion. Neither transfusion nor congenital malaria is expected to relapse because exoerythrocytic schizogony does not occur. The number of cases of malaria reported in the United States averages about 1700 cases per year (CDC, 2018a). The most recent surveillance summary from the CDC reported 1517 total cases in 2015 (Mace et al., 2018). Species causing infection were *P. falciparum* (67.4%), *P. vivax* (11.7%), *P. ovale* (4.1%), *P. malariae* (3.1%), and undetermined (12.9%), with less than 1% having infection with two species. Of reported cases, 17.1% were associated with severe disease, including the 11 persons who died. Only 4.7% of patients with malaria reported adhering to a CDC-recommended chemoprophylaxis malaria regimen. Patients acquired the infection in Africa (84.7%), Asia (8.6%), the Western Hemisphere (4.6%), or Oceania (2 cases). In patients for whom the reason for travel was known, the majority (68.4%) were visiting friends and relatives (VFRs). These individuals have long been known to be at higher risk for acquiring malaria than other travelers since they often do not seek pretravel counseling, do not take malaria chemoprophylaxis, travel to more rural areas, and often stay for longer periods of time.

Clinical Disease

Most patients who develop *P. falciparum* infection become symptomatic within 1 month of exposure, whereas a delay of up to 6 months or more may be seen with the other *Plasmodium* species. Common presenting symptoms of malaria include chills and fever, which often are associated with splenomegaly. In the early stages of the disease, febrile episodes occur irregularly but eventually become more synchronous, assuming the usual tertian (*P. vivax*, *P. falciparum*, and *P. ovale*) or quartan (*P. malariae*) periodicity. Patients with malaria may develop anemia and may have other manifestations, including diarrhea, abdominal pain, headache, and muscle aches and pains. *Plasmodium falciparum* malaria can result in high rates (50%) of parasitemia, which can lead to severe hemolysis with hemoglobinuria and profound anemia. Erythrocytes infected with growing trophozoites and schizonts of *P. falciparum* become sequestered in small vessels of the body, which may lead to occlusion of these vessels, causing symptoms related to capillary obstruction and tissue anoxia. Involvement of the brain is known as cerebral malaria, in which the patient becomes disoriented, progressing to delirium, coma, and often death.

The course of untreated malaria depends on the species. Most fatal cases of malaria are due to *P. falciparum*, although *P. knowlesi* can also cause fatalities. In nonfatal cases, the febrile paroxysms become less severe with time and the disease gradually subsides. Patients with *P. vivax* or *P. ovale* infection may have relapses after many months or, occasionally, years. Persons with *P. falciparum* and *P. malariae* infection may have symptom-free periods but suffer from sporadic recrudescences owing to persisting low-grade parasitemia. Relapses and recrudescences may be associated with changes in the host's defense mechanisms or possibly with antigenic changes in the infecting organisms.

Peripheral smears may show leukocytes that contain malaria pigment (hemozoin). Increased reticulocyte counts occur commonly and are associated with rapid erythrocyte turnover. The presence of greatly enlarged platelets may be noted on peripheral blood films and may occur as a result of their rapid turnover secondary to splenic sequestration. Malarial infection may interfere with certain serologic tests, producing false-positive results, especially those for syphilis.

Therapy and prophylaxis of malaria have become highly complex topics because of the widespread appearance of resistance by *P. falciparum* to chloroquine and other antimalarials and, to a lesser extent, resistance by *P. vivax* to chloroquine. Artemisinin combination therapy is now recognized as the preferred treatment by the WHO for treatment of *P. falciparum* infection and chloroquine-resistant *P. vivax* infection (WHO, 2018). Persons with *P. vivax* or *P. ovale* malaria should receive treatment with primaquine phosphate or tafenoquine in addition to standard primary treatment in order to eradicate hepatic hypnozoites and to prevent relapse. Use of primaquine or tafenoquine may be dangerous in patients who have G6PD deficiency. Screening of at-risk patients before therapy is initiated may be necessary (Drugs for Parasitic Infections, 2013; CDC, 2018a). Exchange transfusion may decrease morbidity and mortality in severe cases of *P. falciparum* infection in which the parasitemia is ≥10% (Pritt, 2019). While exchange transfusion is no longer recommended in this setting by the CDC (Tan et al., 2013), it is still supported in cases of severe malaria with high parasitemia by the American Society for Apheresis (Shaz et al., 2014).

Diagnosis

Malaria should always be included in the differential diagnosis of fever in patients who have a history of travel to or residence in endemic areas (Mathison & Pritt, 2017). Given the potentially life-threatening nature of infection, testing must be performed on a STAT basis. Diagnosis usually is established by demonstrating parasites in thick and thin blood films. Blood specimens ideally are collected just before the next anticipated fever spike or at the onset of fever. Specimens drawn several hours apart sometimes may be required to demonstrate infection or to diagnose the species because the number and morphologic stage of parasites vary during the cycle. Careful examination of thick films should reveal the presence of the parasites in almost all patients with clinically apparent malaria.

Identification of malarial parasites on thin blood films requires a systematic approach. Three major factors should be considered: appearance of infected erythrocytes, appearance of parasites, and stages found. Table 65.7 summarizes diagnostic characteristics of the species, which are illustrated in Figures 65.5 and 65.6. The size of the infected erythrocyte is a particularly useful feature for determining the infecting species; erythrocytes infected by *P. vivax* or *P. ovale* parasites often appear enlarged compared with adjacent uninfected cells, whereas *P. malariae* and *P. falciparum* parasites are usually found in erythrocytes of normal size. Erythrocytes infected with *P. ovale* are often oval or fimbriated (having irregular, usually polar or bipolar, projections of the cell margins), a feature rarely observed with *P. vivax*. Schüffner stippling, noted as numerous small uniform pink granules in the erythrocyte, is usually seen in cells infected with *P. vivax* and *P. ovale*, although it may not be evident in cells infected with early ring forms or on slides that have not been stained at the appropriate pH, such as the Wright-Giemsa stain, used widely in hematology laboratories (see the Laboratory Methods section earlier in the chapter). The presence of Schüffner stippling is helpful because it is not seen in *P. malariae* or *P. falciparum* infection.

As trophozoites grow in the infected cells, the amount of hemoglobin in the erythrocyte decreases and hemozoin pigment accumulates. The amount and appearance of the pigment vary among species. Ring forms of all parasites may have a similar appearance, and if only occasional ring forms are found, the species may not be identifiable. Young rings of *P. falciparum* tend to be smaller than those of the other species (one-sixth the diameter of the red blood cell compared with one-third the diameter of the red blood cell for the other species). Rings of *P. falciparum* that have grown are similar in size to those of the other species. Trophozoites that appear to be lying on the surface of the erythrocyte or protruding from it are called *appliqué* or *accolé* forms, most often seen in *P. falciparum* infection. Doubly infected cells and double chromatin dots in ring trophozoites occur most commonly in *P. falciparum* infection but can occur with the other species as well.

TABLE 65.7
Comparison of *Plasmodium* Species Affecting Humans

Species	Erythrocyte Size	Cytoplasmic Inclusions*	Parasite Cytoplasm	Parasite Pigment	Number of Merozoites	Stages Found in Circulating Blood
Plasmodium vivax	Enlarged; maximum size (attained with mature trophozoites and schizonts) may be 1–2 times normal erythrocyte diameter	Schüffner dots; all stages except early ring forms	Irregular and pleomorphic, ameboid in trophozoites; may have band forms	Golden brown	12–24; average is 16	All stages; wide range of stages may be seen on any given film
Plasmodium malariae	Normal to small	Ziemann dots rarely seen	Rounded, compact trophozoites with dense cytoplasm; band-form trophozoites occasionally seen	Dark brown, coarse, conspicuous	6–12; average is 8; "rosette" schizonts occasionally seen	All stages; wide range of stages usually not seen; relatively few rings or gametocytes generally present
Plasmodium ovale	Enlarged; maximum size may be 1.25 to 1.5 times normal red blood cell diameter; infected red blood cells may be oval and/or fimbriated (border has irregular projections)	Schüffner dots; all stages except early ring forms	Rounded, compact trophozoites; occasionally slightly ameboid; growing trophozoites have large chromatin mass	Dark brown, conspicuous	6–14; average is 8	All stages
Plasmodium falciparum	Normal; multiply infected red blood cells are common	Maurer clefts occasionally seen	Young rings are small, delicate, often with double chromatin dots; gametocytes are crescent or elongate	Black; coarse and conspicuous in gametocytes	6–32	Rings and/or gametocytes; other stages develop in blood vessels of internal organs but are usually not seen in peripheral blood except in severe infection

Modified from Ash LR, Orihel TC: *Atlas of human parasitology,* ed 5, Chicago, 2007, ASCP Press.
*A pH of 7.0–7.2 is optimal for visualization of cytoplasmic inclusions.

Growing trophozoites of *P. vivax* have irregular shapes and are termed *ameboid*. Those of *P. malariae* and *P. ovale* tend to remain compact, although vacuolated forms can occur (with *P. malariae*, these can take on a characteristic shape called the "basket-form" trophozoite). Mature trophozoites and schizonts of *P. falciparum* are usually sequestered in capillary beds secondary to cytoadherence to endothelial cells and are not seen in the peripheral blood except in very severe cases of infection. When schizonts are identified in the peripheral blood, determining the number of merozoites is helpful in identifying the various species. Gametocytes of *P. falciparum* are readily identified by their characteristic sausage shape. Gametocytes of *P. vivax*, *P. malariae*, and *P. ovale* have a similar shape. Thus, they are difficult to differentiate, although characteristics of infected red blood cells can aid in identification.

The varieties of developmental stages in the peripheral blood aid in diagnosis. In *P. falciparum* infection, ring forms predominate; finding numerous ring forms without more mature stages is highly suggestive for *P. falciparum* infection. In *P. vivax*, *P. malariae*, and *P. ovale* infections, various stages of parasites are found with some predominance of one stage depending on the phase of the cycle.

Thick films are preferred for detecting malarial infections because a greater quantity of blood is examined (see the Laboratory Methods section earlier in the chapter). Ring forms often have the appearance of punctuation marks rather than complete rings, and the presence of red chromatin and blue cytoplasm should be required to identify them as parasites (see Fig. 65.6A). Schüffner stippling still may be a helpful identifying characteristic, which may be recognized around growing trophozoites as a pink halo rather than as distinct granules seen in thin films. The ameboid character of *P. vivax* trophozoites is not as evident in thick films, but the number of merozoites in mature schizonts is helpful. Gametocytes usually cannot be differentiated. The distinctive sausage shape of *P. falciparum* gametocytes is still evident, although they may appear stubbier than in thin films. Gametocytes of the other species can be detected and are easily differentiated from host cell nuclei by the presence of refractile hemozoin pigment.

Mixed infections occur occasionally, but caution should be used in making such a diagnosis unless definite evidence reveals two separate populations of parasites. The most common mixed infections are *P. falciparum* and *P. vivax*. Finding gametocytes of *P. falciparum* in a person obviously infected with *P. vivax* is diagnostic, for example.

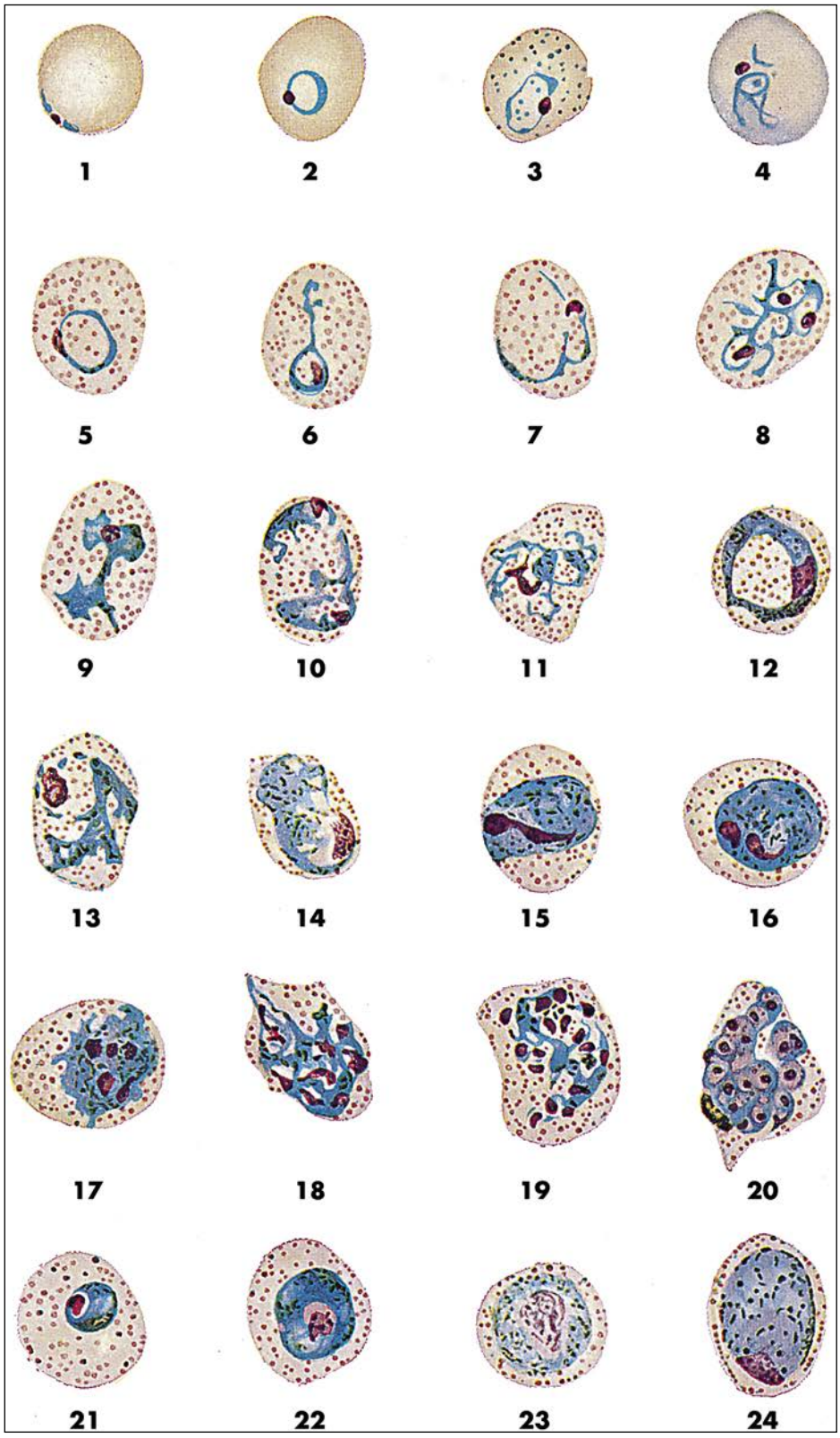
Multiple artifacts may be confused with malarial parasites on thick and thin films. The most common artifacts on thin films are blood platelets superimposed on red blood cells. These platelets should be readily identified because they do not have a true ring form, do not show differentiation of the chromatin and cytoplasm, and do not contain pigment. Clumps of bacteria or platelets may be confused with schizonts. At times, masses of fused platelets may resemble gametocytes of *P. falciparum* but do not show the differential staining or the pigment. Precipitated stain and contaminating bacteria, fungi, or spores may also be confused with these parasites.

Species-specific serologic tests for malaria are not useful for diagnosis of acute infection but may be useful for epidemiologic surveys and detection of infected blood donors (Mathison & Pritt, 2017). Such tests do not reliably differentiate current from past infection, however. Sensitive and specific IFA tests using antigens from the four human species are available from the CDC. Assays for the direct detection of malarial antigens in blood are especially useful (see the Laboratory Methods section earlier in the chapter) (Mathison & Pritt, 2017).

BABESIA SPP. (BABESIOSIS)

Similar to malarial parasites, etiologic agents of babesiosis are apicomplexan protozoa found nearly worldwide that infect erythrocytes, often producing febrile illness of variable severity. Unlike malaria, babesiosis is transmitted by ticks, is found in a variety of animal species that serve as reservoirs, and is primarily a disease of temperate regions. Less commonly, babesiosis is transmitted by blood transfusion and transplacentally.

Human infection in the United States occurs predominantly in the northeastern and Midwestern states, where the rodent parasite *Babesia microti* is responsible for infection. The *Ixodes scapularis*-complex, the "black-legged" or "deer ticks," are the usual vectors. *Babesia duncani* causes a smaller number of infections in northern California, Oregon, and Washington and is thought to be transmitted by the Western black-legged tick, *Ixodes pacificus* (Pritt, 2019). In Europe, babesiosis is caused primarily by *Babesia divergens*, with a smaller number of cases attributed to *Babesia venatorum* and *B. microti*. Infection with these agents is transmitted by *Ixodes ricinus*. Reports of *B. divergens* and *B. divergens*–like (*Babesia* MO-1) infections in the states of Kentucky, Missouri, and Washington expand the range of known human cases in the United States (Herwaldt et al., 2004). Other means of acquiring infection include blood transfusion and, rarely, congenital transmission. The FDA has approved the Imugen (Oxford Immunotec, Oxfordshire, UK) *Babesia microti*–arrayed fluorescent immunoassay for



A

Figure 65.5 **A,** *Plasmodium vivax. 1,* Normal-size erythrocyte with marginal ring-form trophozoite. *2,* Young ring form (early trophozoite) in an enlarged erythrocyte (macrocyte). *3,* Slightly older ring form trophozoite in macrocyte showing Schüffner stippling. *4,* Polychromatophilic macrocyte containing late-stage trophozoite with pseudopodia (i.e., "ameboid" form). *5,* Ring form of trophozoite showing pigment in macrocyte with Schüffner stippling. This stippling does not appear in all cells containing the growing and older forms of *Plasmodium vivax*, but it can be found with any stage from the fairly young ring form onward. *6* and *7,* Ameboid trophozoite forms. *8,* Three ameboid trophozoites. *9* to *13,* Older ameboid trophozoites in the process of development. *10,* Two ameboid trophozoites in one cell. *14,* Mature trophozoite. *15,* Mature trophozoite with chromatin apparently in process of division. *16* to *19,* Schizonts showing progressive steps in division (early segmenting schizonts). *20,* Mature schizont. *21* and *22,* Developing gametocytes. *23,* Mature microgametocyte. *24,* Mature macrogametocyte. (From Wilcox A: *Manual for the microscopical diagnosis of malaria in man,* Bulletin No. 180, Bethesda, MD, 1942, National Institutes of Health.)

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B

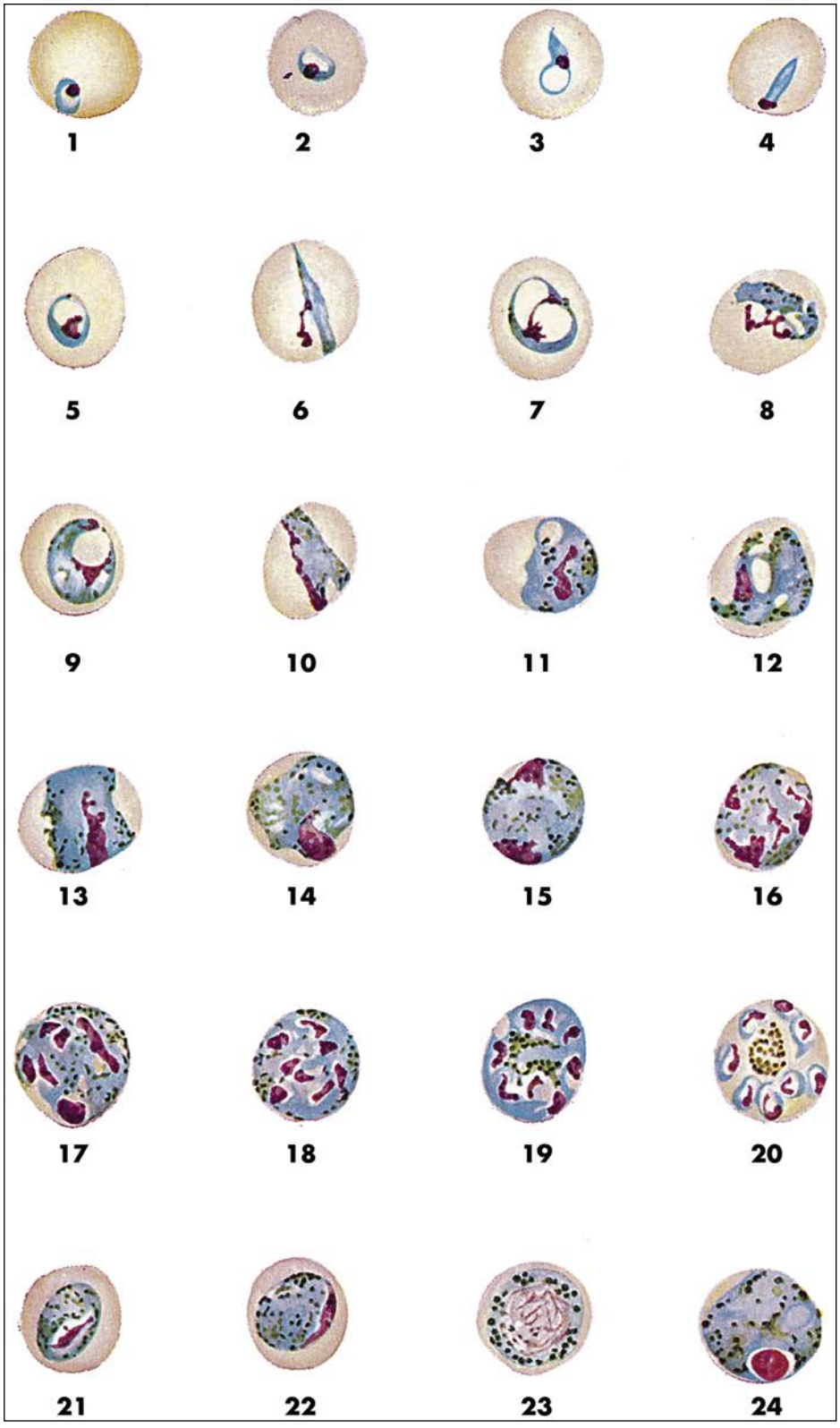

Figure 65.5—cont'd **B,** *Plasmodium malariae. 1,* Young ring form (early trophozoite). *2* to *4,* Young trophozoite forms of the parasite showing gradual increase in chromatin and cytoplasm. *5,* Developing ring form of trophozoite showing pigment granule. *6,* Early band form of trophozoite—elongate chromatin, some pigment apparent. *7* to *12,* Some forms that the developing (late-stage) trophozoite of *P. malariae* may take. *13* and *14,* Late-stage trophozoites—*13* shows a thick band form. *15* to *19,* Phases in the development of the schizont (early segmenting schizonts). *20,* Mature schizont containing a central hemozoin pigment granule and peripheral merozoites ("rosette" or "daisy head" arrangement). *21,* Immature microgametocyte. *22,* Immature macrogametocyte. *23,* Mature microgametocyte. *24,* Mature macrogametocyte. (From Wilcox A: *Manual for the microscopical diagnosis of malaria in man,* Bulletin No. 180, Bethesda, MD, 1942, National Institutes of Health.)

C

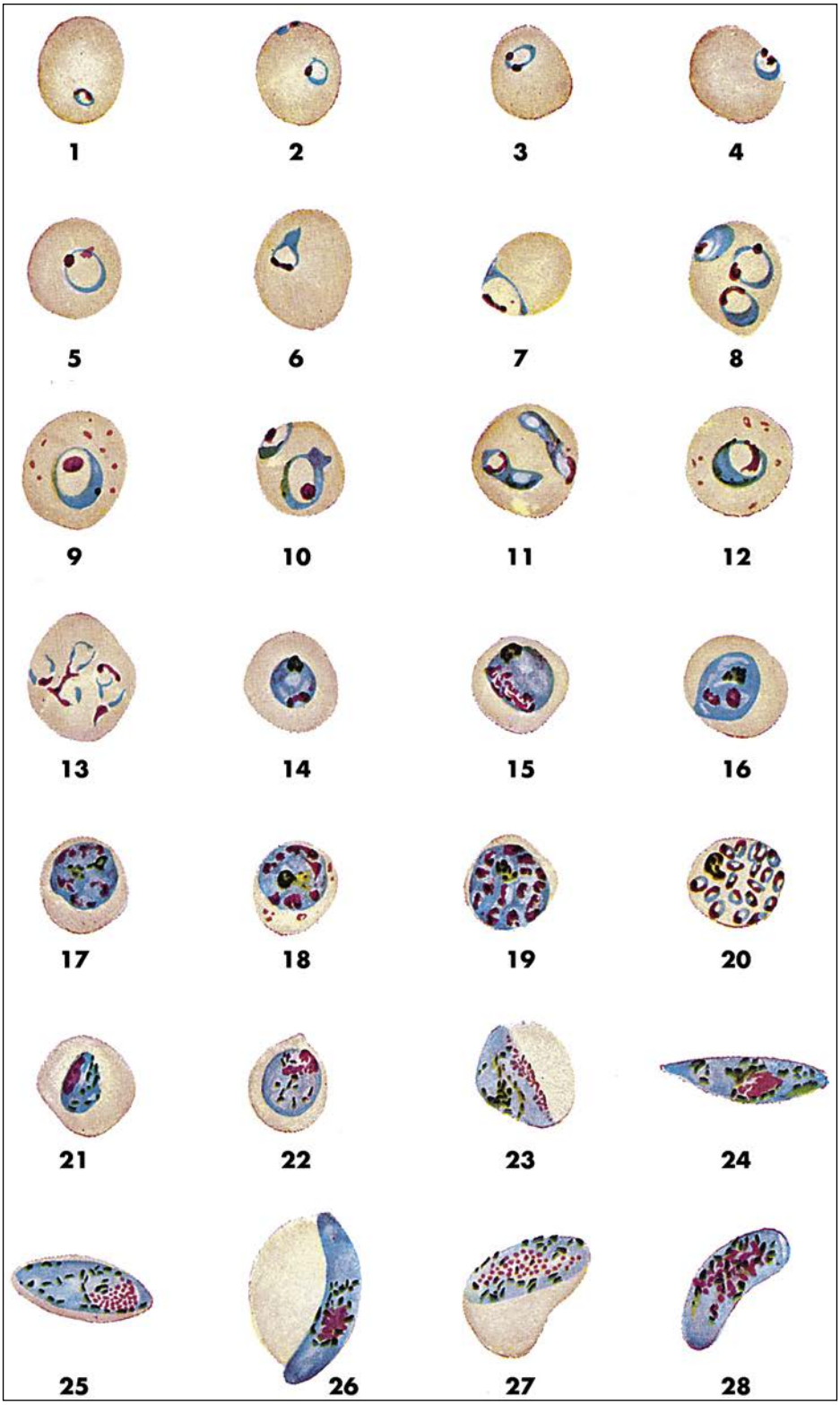

Figure 65.5—cont'd **C,** *Plasmodium falciparum. 1,* Very young ring form (early trophozoite). *2,* Double infection of single cell with young trophozoites—one a "marginal" or "applique" form, the other a "signet ring" form. *3* and *4,* Young trophozoites showing double chromatin dots ("headphone" forms). *5* to *7,* Developing trophozoite forms. *8,* Three late-stage trophozoites in one cell. Although these forms are not generally seen in freshly obtained peripheral blood with *P. falciparum* infection due to sequestration in the peripheral capillaries, they may be seen when there is a delay between collection and examination. *9,* Trophozoite showing pigment in a cell containing Maurer dots. These intracytoplasmic inclusions can be differentiated from the stippling seen with *P. vivax* and *P. ovale* infection in that the Maurer dots are larger and fewer in number. *10* and *11,* Two late-stage trophozoites in each of two cells, showing variation in forms that parasites may assume. *12,* Late-stage trophozoite showing haze of pigment throughout cytoplasm. Maurer dots are also present. *13,* Unusual slender forms of *P. vivax*. *14,* Mature trophozoite, showing clumped pigment. *15,* Parasite in the process of initial chromatin division to form a schizont (schizonts are not usually found in the peripheral circulation in *P. falciparum* infection). *16* to *19,* Various phases of development of the schizont (early segmenting schizonts). *20,* Mature schizont. *21* to *24,* Successive forms in development of the gametocyte—usually not found in the peripheral circulation. *25,* Immature macrogametocyte. *26,* Mature macrogametocyte. *27,* Immature microgametocyte. *28,* Mature microgametocyte. (Courtesy of United States Public Health Service, National Institutes of Health.)

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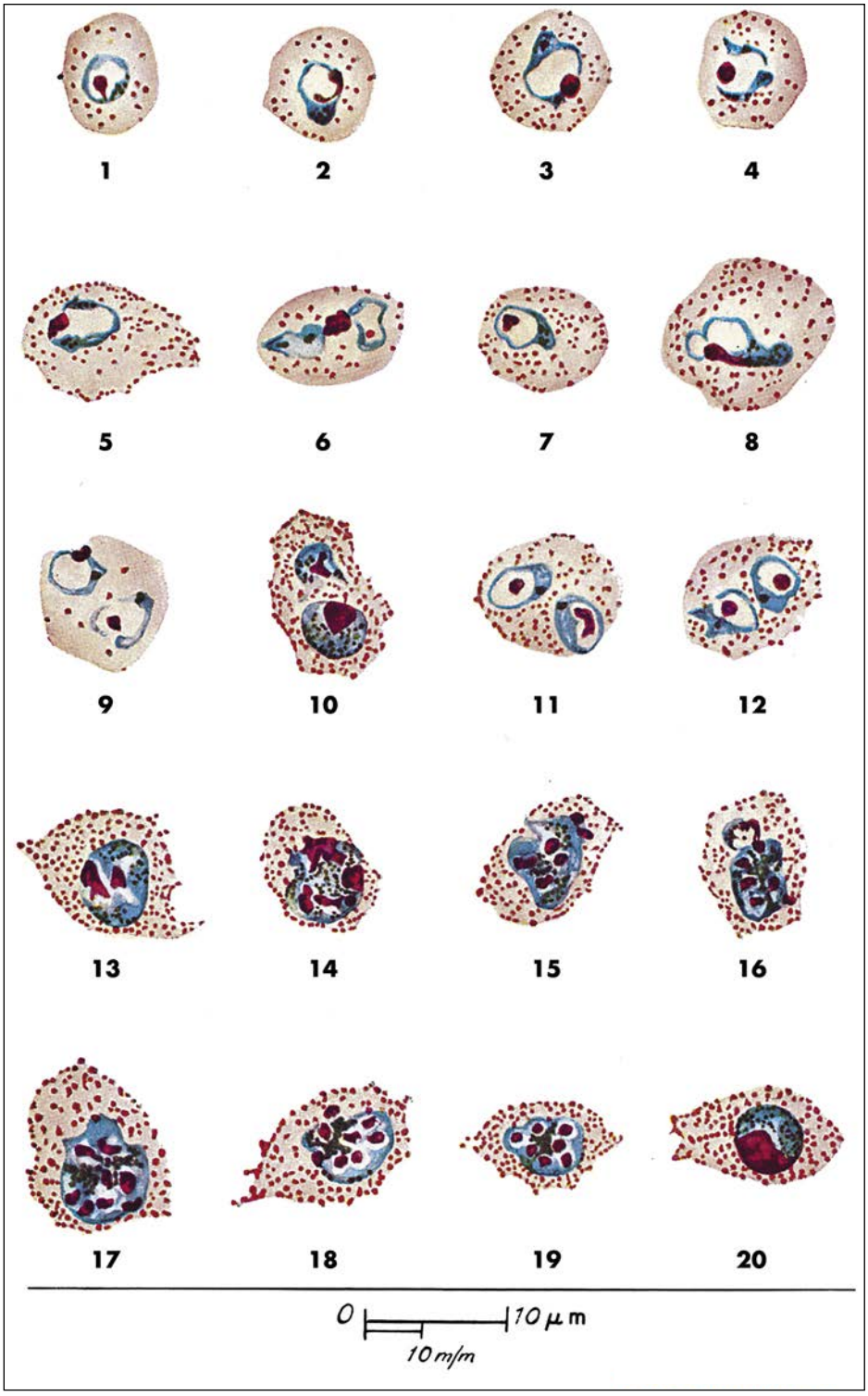


D

Figure 65.5—cont'd **D,** *Plasmodium ovale. 1,* Young ring form (early trophozoite). *2* to *5,* Older ring-shaped trophozoites. *6* to *8,* Older trophozoites, some with ameboid features reminiscent of *P. vivax* infection. Generally, *P. ovale* late-stage trophozoites have a more compact appearance than those of *P. vivax. 9, 11,* and *12,* Doubly infected cells, trophozoites. *10,* Doubly infected cell, young gametocytes. *13,* Early segmenting schizont with only 2 merozoites. *14* to *19,* Schizonts, progressive stages. *20,* Mature gametocyte. (Free translation of legend accompanying original plate in *Guide pratique d'examen microscopique du sang appliqué au diagnostic du paludisme* by Georges Villain. Reproduced with permission from Biologie Medicale Supplement, 1935. Courtesy of Aimee Wilcox, National Institutes of Health Bulletin No. 180, United States Public Health Service.)

detecting *B. microti* antibodies in human plasma samples as well as the Imugen *Babesia microti* nucleic acid test for detection of DNA in whole blood (https://www.fda.gov/news-events/press-announcements/fda-approves-first-tests-screen-tickborne-parasite-whole-blood-and-plasma-protect-us-blood-supply).

The spectrum of babesiosis varies from latent, subclinical infection to fulminant, hemolytic disease. Fatalities have been reported, especially in splenectomized or immunocompromised individuals. Immunocompetent persons may experience symptoms similar to those of malaria, including fever, chills, malaise, and anemia, although without recognizable periodicity. Investigation of an outbreak caused by *B. microti* on Nantucket Island in New England showed that some symptomatic patients harbored the parasite for months and others showed serologic evidence of infection without a history of clinical disease. Other evidence indicates that chronic subclinical infections may not be uncommon (Westblade et al., 2017). While asymptomatic infections in immunocompetent patients generally do not require treatment, symptomatic patients, including those at risk for severe disease (e.g., asplenic individuals), are generally treated using atovaquone plus azithromycin or clindamycin plus quinine (Drugs for Parasitic Infections, 2013).

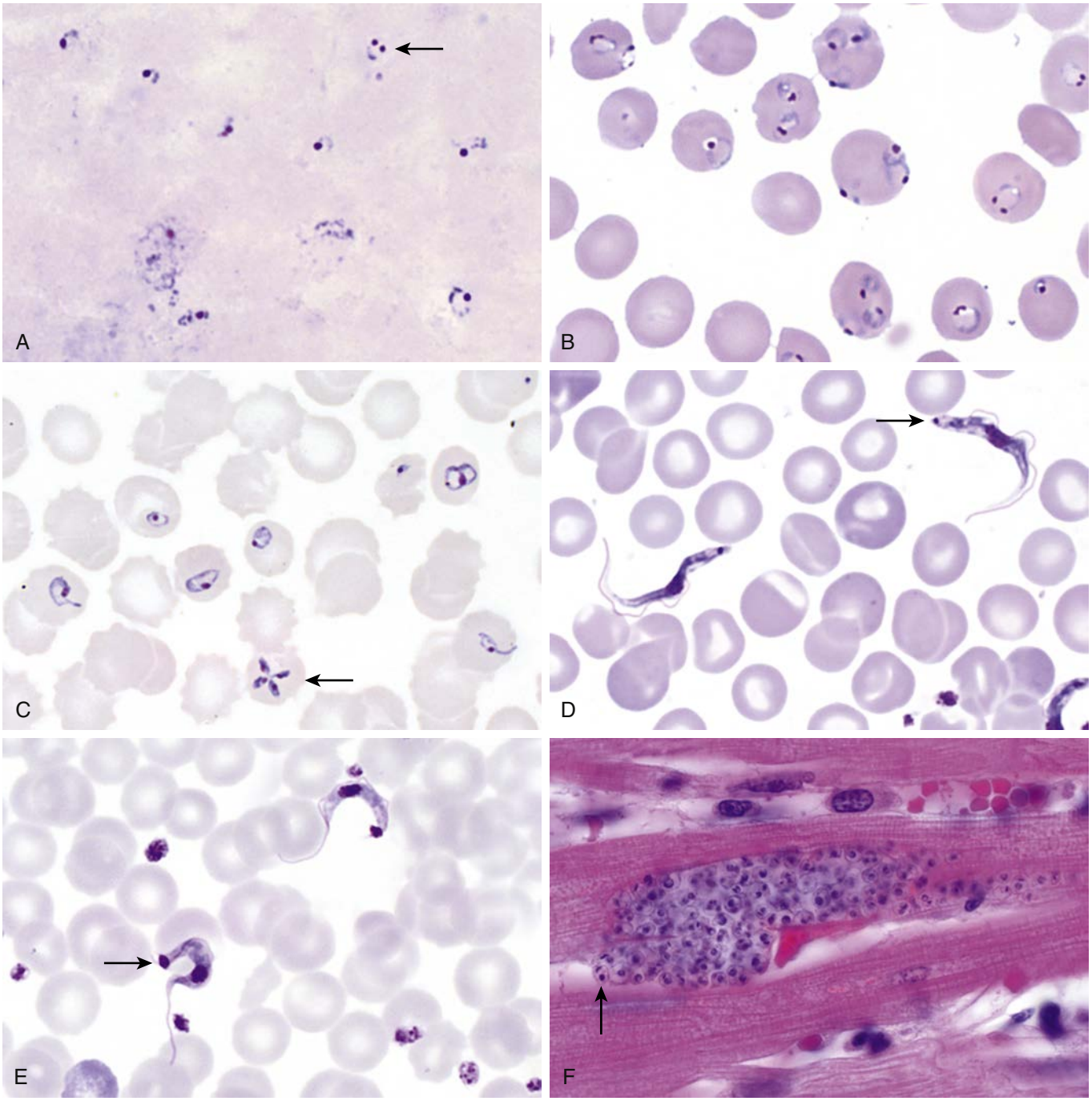


Figure 65.6 **A,** *Plasmodium falciparum* in Giemsa-stained thick blood film showing thin, delicate rings and a "headphone" form *(arrow)* (1000×). **B,** *Plasmodium falciparum* in Giemsa-stained thin blood film; note high parasitemia, multiply infected red blood cells, and delicate ring forms with appliqué and headphone forms (1000×). **C,** *Babesia microti* in Giemsa-stained thin blood film; note high parasitemia, multiply infected red blood cells with irregular forms, and Maltese cross configuration *(arrow)* (1000×). **D,** *Trypanosoma brucei* in thin blood film; note nucleus, small kinetoplast *(arrow)*, anterior flagellum, and undulating membrane (Giemsa stain; 1000×). **E,** *Trypanosoma cruzi* in thin blood film; note nucleus, large kinetoplast *(arrow)*, anterior flagellum, and undulating membrane (Giemsa stain; 1000×). **F,** *Trypanosoma cruzi* amastigotes in a cardiac biopsy; individual amastigotes with a small nucleus and rod-shaped kinetoplast *(arrow)* are visible (hematoxylin and eosin [H&E], 1000×).

Babesia parasites multiply in erythrocytes by binary fission, producing morphologically indistinguishable trophozoites and gametes. Although trophozoites of many species may be highly variable in size and shape, those of *B. microti* usually appear as delicate ring forms that may be easily confused with those of malarial parasites, especially *P. falciparum* (see Fig. 65.6C) (Westblade et al., 2017). *Babesia* can be differentiated from those of malarial parasites by the presence of a tetrad (Maltese cross) formation of the merozoites and the absence of large ameboid trophozoites and morphologically distinguishable gametocytes; extracellular forms may be seen in heavy infections. Also, *Babesia* species usually have a heterogeneous appearance with round, oval, spindled, and "racket" forms coexisting on the same peripheral blood smear. Finally, *Babesia*-infected cells lack hemozoin pigment, which is present in *Plasmodium*-infected cells. History of residence in or travel to endemic areas, or of a recent tick bite, might suggest *Babesia* infection. NAATs for *Babesia* species are available from the CDC on referral from state health departments and from some commercial laboratories. Serologic tests (e.g., IFA) may also be available but are generally not useful for detection of acute disease. However, they may be useful for screening blood donors in endemic settings. Serology tests for malaria are negative in babesiosis, although patients with malaria may cross-react in the *Babesia* serologies (Westblade et al., 2017; Pritt, 2019).

HEMOFLAGELLATES

The hemoflagellates of humans and animals are members of the group *Kinetoplastea* and are characterized by the presence of a kinetoplast, a complex of abundant circular DNA within a large mitochondrion, which can be seen by light microscopy when treated with Giemsa stain. Two genera

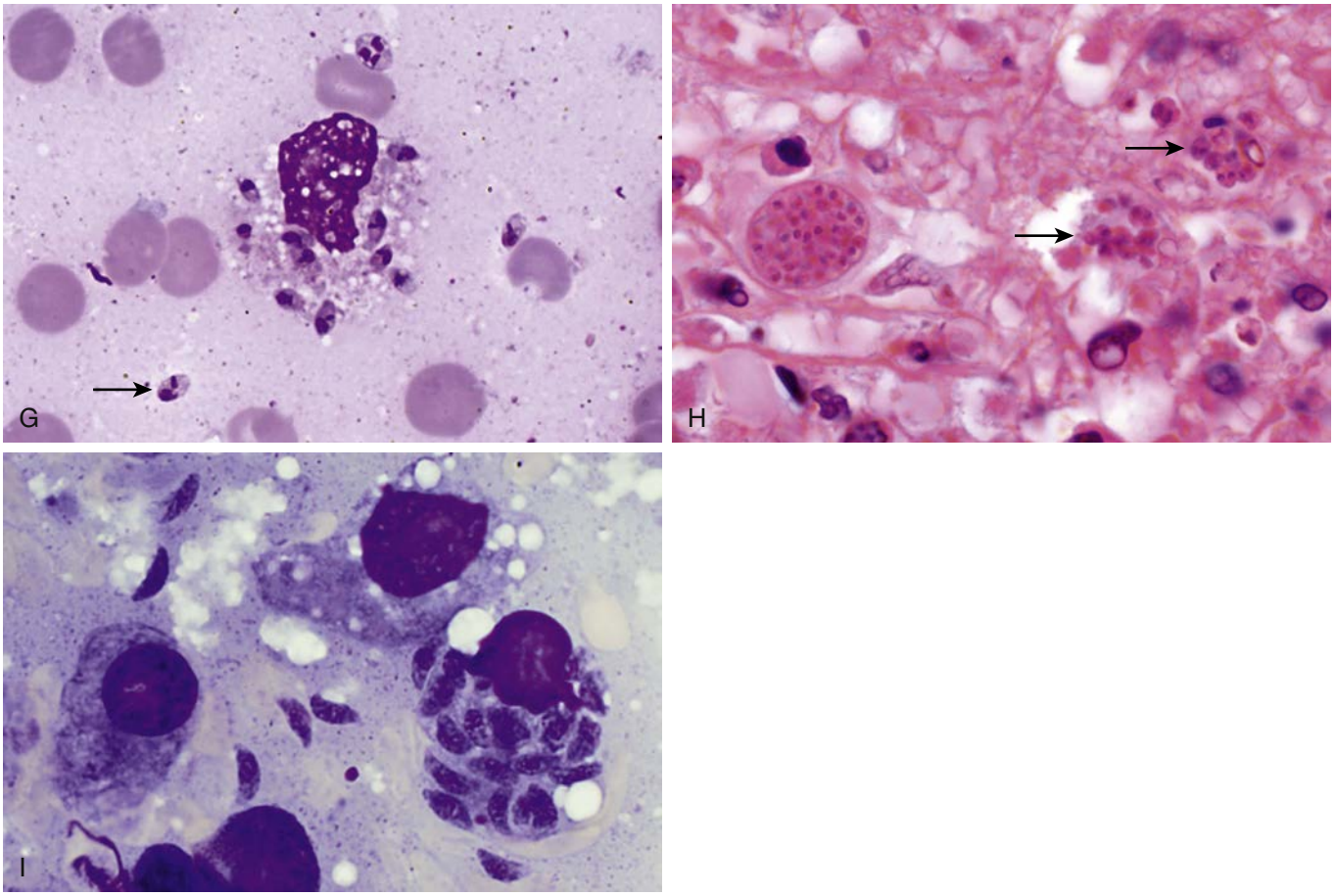


Figure 65.6—cont'd **G,** *Leishmania* sp. amastigotes in an impression smear of lymph node (Giemsa stain; 1000×). **H,** Pseudocyst *(left)* and free tachyzoites *(arrows)* of *Toxoplasma gondii* in brain tissue (H&E; 1000×). **I,** Intracellular and free *T. gondii* tachyzoites in an impression smear of brain tissue from an immunocompromised patient with reactivated disease (Giemsa; 1000×).

important in human disease are *Trypanosoma* and *Leishmania*. Members of both genera are transmitted by arthropod vectors and have animal hosts that serve as reservoirs.

Kinetoplastids assume different morphologic forms depending on their presence in vertebrate hosts, including humans, or in their insect vectors (Fig. 65.7). The amastigote stage is spherical, 2 to 5 μm in diameter, and displays a nucleus and kinetoplast. By definition, an external flagellum is lacking, although an axoneme (the intracellular portion of the flagellum) is apparent at the ultrastructural level. Amastigotes may be found in human or animal hosts infected with *T. cruzi* or *Leishmania* spp., where they multiply exclusively within cells, but not with *T. brucei*. The promastigote is an elongated and slender organism with a central nucleus, an anteriorly located kinetoplast and axoneme, and a free flagellum extending from the anterior end. This stage occurs in the insect vectors of *Leishmania* and is the stage detected in culture but is not detected in clinical specimens unless there is a substantial delay in processing. The epimastigote is similar to the promastigote, but the kinetoplast is found closer to the nucleus and has a small undulating membrane that becomes a free flagellum. All species of *Trypanosoma* that infect humans assume an epimastigote stage in the insect vector or in culture. Neither the promastigote nor epimastigote are seen in humans. In the trypomastigote, the kinetoplast is found at the posterior end and the flagellum forms an undulating membrane that extends the length of the cell, emerging as a free flagellum at the anterior end. Trypomastigote forms occur predominantly in the bloodstream of mammalian hosts infected with various *Trypanosoma* spp. Infectious stages found in appropriate insect vectors following transformation from the epimastigote form are known as *metacyclic trypomastigotes*.

Trypanosoma spp. (African and American Trypanosomiasis)

Infections with trypanosomes include those caused by *Trypanosoma brucei* (African trypanosomiasis) and *T. cruzi* (American trypanosomiasis, or Chagas disease). Both are of great importance in endemic areas. A third species, *Trypanosoma rangeli*, has been described in humans in the Americas but does not cause clinical illness. Bloodstream trypomastigotes of the *T. brucei* group (see Fig. 65.6D) are up to 30 μm long with graceful curves and a small kinetoplast. Those of *T. cruzi* are somewhat shorter (20 μm) and display a larger kinetoplast. Also, dividing forms may be seen in blood films with *T. brucei* but not *T. cruzi*, as the latter replicates only in the amastigote stage in the human host.

African Trypanosomiasis

In equatorial Africa, parasites of the *T. brucei* group infect both animals and humans and are transmitted by the bite of tsetse flies in the genus *Glossina*. Multiplication of organisms at the bite site often produces a transient chancre. East African trypanosomiasis is caused by *T. brucei rhodesiense*, which has a number of animal reservoir hosts. The disease is characterized by a rapidly progressive acute febrile illness with lymphadenopathy. Patients may die before central nervous system (CNS) involvement is prominent.

The infection in western Africa is caused by *T. brucei gambiense*, which is responsible for classic African sleeping sickness. The disease has a more chronic course that begins with intermittent fevers, night sweats, and malaise. Lymphadenopathy, especially of the posterior cervical lymph nodes (Winterbottom sign), may be pronounced. Involvement of the CNS becomes prominent with time. Somnolence, confusion, and fatigue progress, leading to stupor, coma, and eventual death. Humans are the primary reservoir for this disease (Bruckner & Labarca, 2019).

Treatment varies by stage of infection (hemolymphatic vs. late disease with CNS involvement) and is potentially toxic. The organoarsenic compound melarsoprol is the only drug available for treating *T. b. rhodesiense* late-stage disease and carries an associated 1% to 5% mortality rate (Drugs for Parasitic Infections, 2013; WHO, 2019). Consultation with the CDC or other public health experts is indicated in nonendemic settings.

The diagnosis is suspected on the basis of geographic history and clinical findings. Patients show high total IgM levels in blood and cerebrospinal fluid (CSF). Pleocytosis occurs with 50 to 500 mononuclear cells per microliter in CSF. The diagnosis is established by demonstrating the parasites on thick and thin films of peripheral blood, buffy coat preparations, or aspirates of lymph nodes or bone marrow, or in spun CSF that is stained with Giemsa (Bruckner & Labarca, 2019). Culture or animal inoculation may be helpful if it is available; a number of molecular methods have also been described.

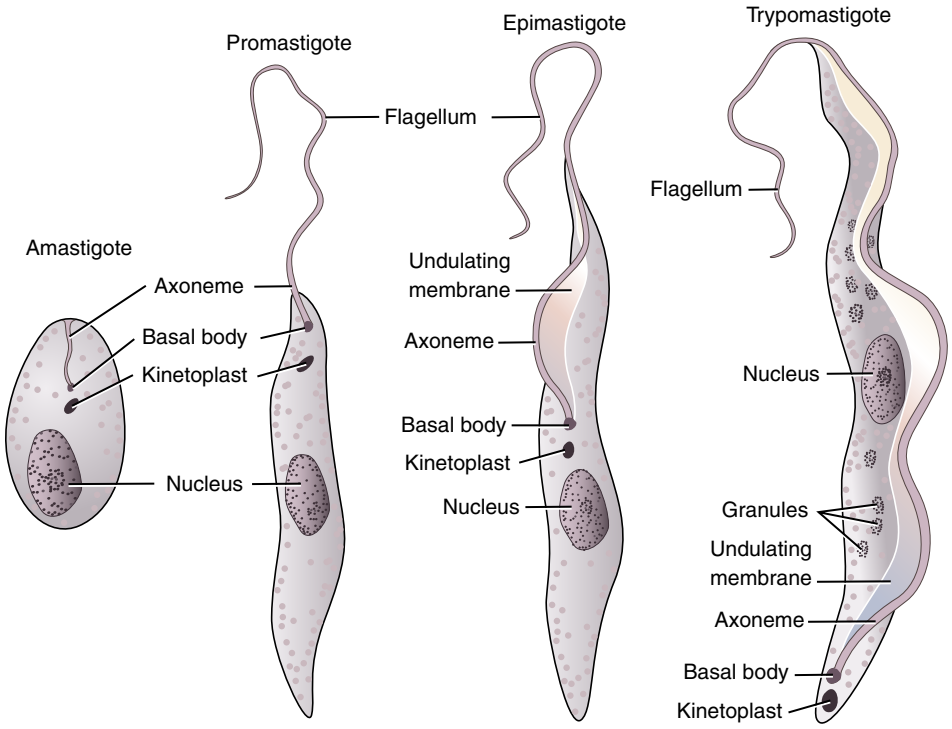


Figure 65.7 Morphology of hemoflagellates commonly infecting humans.

American Trypanosomiasis

American trypanosomiasis, or Chagas disease, is caused by *T. cruzi*. In its sylvatic form, the parasite occurs in the United States, Mexico, Central America, and most of South America. Human infections are common in parts of Mexico and Central and South America, where they are transmitted by kissing bugs in the family Reduviidae. In contrast, only rare cases of locally acquired Chagas have been documented in the United States. However, Chagas is now recognized as an important parasite in the United States due to the large number of immigrants that the country receives from endemic areas. As such, it has been designated as an NPI targeted for public health action (CDC, 2018b). The CDC and WHO estimate that more than 8 million individuals are infected worldwide and that greater than 300,000 infected individuals currently live in the United States (CDC, 2018b).

Genera and species of reduviid bugs involved in transmission vary from one country to another and among different ecologic niches. Some reduviids are responsible for maintaining the sylvatic cycle in animal reservoirs, whereas others are adapted to an anthropophilic life in which they infest poorly constructed houses, usually in rural areas. At the time of feeding, the reduviid bug defecates. The bug feces contain infective trypomastigotes that, as a result of scratching or rubbing, enter the body at the bite site or through intact mucosa of the mouth or conjunctiva. Infective forms actively enter nearby tissue cells, where they transform into dividing amastigotes. When the infected cell is filled with amastigotes, transformation to trypomastigotes occurs, followed by cell rupture. Trypomastigotes are released into the peripheral blood and reach distant tissues, where they transform into amastigotes and start the reproductive cycle de novo. Other important means of infection are vertical transmission, blood transfusion, organ transplantation from an infected donor, and, rarely, laboratory accident and through ingestion of contaminated food or drink (CDC, 2018b). Given the risk of transmission via blood transfusion, blood donor units are routinely screened in the United States for *T. cruzi* using FDA-approved serologic tests.

Chagas disease may cause acute or chronic infection. Acute disease is most common in children younger than 5 years of age and is characterized by malaise, chills, fever, hepatosplenomegaly, and myocarditis. Swelling of the tissues around the eye (Romaña sign) may be present if inoculation of the organisms occurs on the face. Swelling of tissues at other locations following the bite of an infected reduviid is called a *chagoma*. In older individuals, the acute course is milder and often asymptomatic, and the patient remains infected for life. Chronic manifestations of the infection, including megaesophagus, megacolon, and alterations in the conduction system of the heart, are related to destruction of the effector cells of the parasympathetic system by autoantibodies. Infection can be transmitted by blood transfusion, and quiescent infections may be exacerbated by immunosuppression.

Diagnosis of Chagas disease can be challenging. The trypomastigotes (Fig. 65.6E) can be observed in peripheral blood or CSF only during the acute stage of the disease or during reactivation. Amastigotes can also be identified in heart biopsies (Fig. 65.6 *F)*, although this method is not commonly employed. Molecular diagnosis may be employed when morphologic diagnosis is inappropriate; examples of such clinical scenarios include (1) a person with a bug bite who has emigrated from or returned from an endemic area within 2 months, (2) monitoring organ transplant recipients after initial serologic testing, (3) laboratory accidents (e.g., accidental inoculation), and (4) suspected congenital cases. Often, more than one molecular assay is performed as different assays have different molecular targets (Qvarnstrom et al., 2012). Diagnosis of chronic Chagas disease is best achieved by antibody detection. Other clinical scenarios that may warrant antibody detection include (1) screening of blood and organ donors, (2) symptomatic patients with appropriate travel or exposure history, (3) initial transplant recipients with appropriate epidemiologic history or who received donated organs from an individual with appropriate epidemiologic history, and (4) possible congenital cases. No single serologic assay is sensitive and specific enough to be relied on alone. Therefore, per current recommended guidelines and the CDC, serologic confirmation of chronic *T. cruzi* infection requires reactivity on two tests using two different methodologies and/or two different *T. cruzi* antigen preparations. When results are discordant, a testing by a third assay is recommended to resolve the initial results or repeat testing on a new sample may be required. The CDC performs an ELISA (Chagatest, Wiener Laboratories, Rosario, Argentina) and a Trypomastigote-Excreted Secreted Antigen (TESA) immunoblot (LDT, CDC) for primary testing and an LDT immunofluorescence assay (IFA) for discordant results. A TESA blot can also be obtained commercially (BioMérieux, Rio de Janeiro, Brazil) (Bern et al., 2007; Afonso et al., 2012). In endemic areas, xenodiagnosis (examination of the gut contents of laboratory-raised reduviids that have been allowed to feed on a patient) may be used. In the chronic stage, serodiagnosis is the method of choice.

Management and treatment of Chagas disease is complex and varies with the age of the patient and stage of infection (CDC, 2018b; Drugs for Parasitic Infections, 2013). Antiparasitic therapy using nifurtimox or benznidazole is indicated for all cases of acute and reactivated disease and is also recommended for cases of chronic infection in children ≤18 years of age. Antiparasitic treatment is also strongly recommended for adults up to 50 years old with chronic disease in which advanced-stage cardiac involvement is not present. The potential benefits and risks of treatment for older individuals and those with advanced cardiomyopathy must be individually weighed prior to offering therapy (CDC, 2018b; Drugs for Parasitic Infections, 2013).

Leishmania spp. (leishmaniasis)

Leishmaniasis is a disease of the reticuloendothelial system caused by kinetoplastid protozoa of the genus *Leishmania*. All species that infect humans have animal reservoirs and are transmitted by sand flies belonging to the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World. The parasites assume the amastigote form in mammalian hosts and the promastigote form in insect vectors. Species of *Leishmania* cannot be differentiated by examination of amastigotes or promastigotes. Leishmaniasis may assume many different clinical forms; cutaneous, mucocutaneous, and visceral diseases are best known. The form and severity of disease vary with the infecting species, the particular host's immune status, and prior exposure (Bruckner & Labarca, 2019).

Cutaneous Leishmaniasis

Old World cutaneous leishmaniasis occurs in southern Europe, northern and eastern Africa, the Middle East, Iran, Afghanistan, India, and southern Russia. Infections are caused by *Leishmania tropica*, *Leishmania major*, and *Leishmania aethiopica*, although *L. donovani* and *Leishmania infantum* may also produce cutaneous lesions. The lesions of cutaneous leishmaniasis may be variable in appearance, with "wet," "dry," and warty appearances. Locally, they may be referred to by a variety of names such as oriental sore, Aleppo boil, desert boil, and Delhi boil. *Leishmania tropica* produces the "urban" dry ulcer, which is more long-lived than the "rural" wet ulcer of *L. major*. Ulcers caused by these species usually develop on an exposed area of the body and heal spontaneously. Infection produces long-lasting immunity. *L. tropica* may become viscerotropic, as was demonstrated in military personnel who participated in Operation Desert Storm (Magill et al., 1993). *Leishmania aethiopica* causes a more aggressive cutaneous infection, which in some individuals metastasizes to produce mucosal lesions or diffuse cutaneous leishmaniasis, the latter of which is characterized by multiple skin nodules resembling lepromatous leprosy.

Cutaneous leishmaniasis of the New World is caused by many species, including *Leishmania mexicana*, *Leishmania braziliensis*, *Leishmania amazonensis*, *Leishmania venezuelensis*, *Leishmania peruviana*, *Leishmania panamensis*, and *Leishmania guyanensis*, among others (Bruckner & Labarca, 2019). Lesions produced by *L. mexicana* often involve the earlobe (Chiclero ulcer), are self-limiting, and are not known to metastasize to the mucosa. However, *L. mexicana* and *L. amazonensis* may produce diffuse cutaneous lesions similar to those produced by *L. aethiopica*. A focus of cutaneous leishmaniasis exists in the southern part of Texas, where infections are caused by one or more species (McIlwee et al., 2018). *Leishmania peruviana*, which has been found on the western slopes of the Peruvian Andes, causes an infection called uta, a benign cutaneous lesion that occurs predominantly in children. *L. peruviana* is acquired in the home, where the main reservoirs are domestic dogs. This epidemiologic situation contrasts with other cutaneous leishmaniases, which usually are acquired in forests and have wild animals as reservoir hosts. Treatment of cutaneous leishmaniasis is based on the causative species and location and extent of disease. In endemic settings outside of the Americas, where there is no risk of mucosal dissemination, cosmetically unimportant lesions may be treated topically (e.g., cryotherapy) or allowed to self-heal. For individuals at risk of aggressive, disfiguring, or disseminated disease, systemic therapies with sodium stibogluconate, meglumine antimoniate, miltefosine, or paromomycin may be used (Drugs for Parasitic Infections, 2013). Topical paromomycin may also be used.

Mucocutaneous (Mucosal) Leishmaniasis

Mucocutaneous leishmaniasis (espundia) is caused primarily by *L. braziliensis* and species in the *Viannia* subgenus, which produce typical cutaneous lesions that generally are more aggressive, last longer, and often disseminate to mucous membranes, especially in the nasal, oral, or pharyngeal areas. In these locations, they may produce disfiguring lesions secondary to erosion of soft tissues and cartilage. *L braziliensis* is distributed in Mexico and Central and South America. Treatment is with sodium stibogluconate, meglumine antimoniate, amphotericin, or miltefosine (Drugs for Parasitic Infections, 2013).

Visceral Leishmaniasis

Visceral leishmaniasis of the Old World occurs sporadically over a wide geographic area and is caused by *L. donovani* or *L. infantum*. *L. donovani* predominates in Africa, India, and Asia, and *L. infantum* (syn. *L. chagasi*) occurs in the Mediterranean region, Middle East, Central Asia, and Central and South America. New World visceral leishmaniasis is caused by *L. chagasi* and occurs sporadically throughout Central and South America. On occasion, some species that cause cutaneous disease have been responsible for visceral disease, as demonstrated in some troops who participated in Operation Desert Storm (Magill et al., 1993). In some areas, humans may serve as the disease reservoir, although a variety of animals, including dogs and cats, usually assume this role.

The infection is usually benign and often subclinical, although some individuals, especially young children and malnourished individuals, have marked involvement of the viscera, especially liver, spleen, bone marrow, and lymph nodes. In some cases, death occurs after months to years unless it is treated appropriately. The infection is called *kala-azar* in India in reference to the darkening of the skin. Treatment is liposomal amphotericin B, sodium stibogluconate, meglumine antimoniate, miltefosine, amphotericin, or paromomycin (Drugs for Parasitic Infections, 2013). Visceral leishmaniasis is an opportunistic infection in individuals with concurrent human immunodeficiency virus (HIV); the condition responds poorly to therapy in such circumstances (Bruckner & Labarca, 2019).

Diagnosis of Leishmaniasis

The diagnosis usually is established by visualization of amastigotes in smears, imprints, or biopsies, or by growth of promastigotes in culture. In integumentary leishmaniasis, the border of the most active lesion should be biopsied and the fresh biopsy should be used to make imprints. The CDC provides instructions for specimen collection at https://www.cdc.gov/parasites/leishmaniasis/resources/pdf/cdc_diagnosis_guide_leishmaniasis_2016.pdf. Both the imprints and smears should be stained with Giemsa. Biopsies should be examined by histopathology and/or submitted for culture. Specimens that may be submitted when visceral leishmaniasis is suspected include buffy coat preparations, lymph node and bone marrow aspirates, spleen and liver biopsies, and antibody detection (Garcia, 2016).

A culture is desirable because it is more sensitive than microscopic examination and allows determination of the species or subspecies, a practice that may help in clinical management of the patient. Biopsy or aspirate specimens collected aseptically are cultured in Novy-MacNeal-Nicolle medium or in Schneider's *Drosophila* medium supplemented with fetal calf serum. Cultures usually begin to show promastigotes in 2 to 5 days but should be held for 4 weeks. The CDC provides culture collection kits after consultation and will perform culture and species identification using PCR and sequencing analysis (de Almeida et al., 2011).

Amastigotes found in imprints, smears, and tissue sections are recognized by their size (2–4 μm) and the presence of delicate cytoplasm, a nucleus, and a kinetoplast (see Fig. 65.6G). In tissue sections, they may appear smaller because of shrinkage during fixation. Amastigotes must be differentiated from other intracellular organisms, including yeast cells of *Histoplasma capsulatum* and trophozoites of *T. gondii*. *Leishmania* spp. have a kinetoplast and do not stain with Gomori methenamine silver (GMS) or periodic acid–Schiff (PAS). In contrast, *Histoplasma* lack the kinetoplast, and the cell wall stains with PAS and GMS. According to one study (Weigle et al., 1987), the sensitivity of histologic sections stained with hematoxylin and eosin (H&E) is 14%; imprints 19%, cultures 58%, and all methods combined, 67%. It should be noted that the amastigotes of *Leishmania* spp. and *T. cruzi* are morphologically indistinguishable.

TOXOPLASMA GONDII (TOXOPLASMOSIS)

Toxoplasma gondii is a protozoan parasite of the Apicomplexa clade that has a worldwide distribution in humans and in domestic and wild animals, especially carnivores. Infection in immunocompetent persons is generally asymptomatic or mild, but immunocompromised patients may experience serious complications. Infection in utero may result in serious congenital infection with sequelae or stillbirth (McAuley & Singh, 2019). The CDC estimates that more than 40 million individuals in the United States alone are infected with this parasite and have identified it as one of five NPIs targeted for public health action (CDC, 2018b).

The sexual stage in the life cycle of this coccidian parasite is completed in the intestinal epithelium of cats and other felines, which serve exclusively as definitive hosts. During this enteroepithelial cycle, asexual schizogony and sexual gametogony occur, leading to the development of immature oocysts that are passed in the feces. Oocysts mature to the infective stage (which contain two sporocysts with four sporozoites each) in the environment in 2 to 21 days. Ingestion of infective oocysts may lead to infection of a wide variety of susceptible vertebrate hosts in which actively growing trophozoites (tachyzoites) may infect any nucleated cells. Proliferation of tachyzoites results in cell death and injury to the host during acute infection. Once immunity has developed, the organisms form tissue cysts that may eventually contain hundreds or thousands of slowly growing bradyzoites. The presence of tissue cysts is characteristic of chronic infection. All stages of the life cycle occur in felines, but only trophozoite and cyst stages occur in humans and other intermediate hosts.

Humans acquire infection with *T. gondii* by ingestion of inadequately cooked meat, especially lamb or pork, that contains tissue cysts or by ingestion of infective oocysts from material contaminated by cat feces. Outbreaks have occurred from inhaling contaminated dust in an indoor riding stable (Teutch et al., 1979) and from drinking contaminated water or unpasteurized goat's milk (Benenson et al., 1982; Sacks et al., 1982; Bowie et al., 1997). Transmission via blood transfusion, organ transplantation, and transplacentally to the developing fetus also can occur.

Most acute infections are asymptomatic or mimic other infectious diseases in which fever and lymphadenopathy are prominent, such as infectious mononucleosis and acute HIV infection. Congenital infection may occur when the mother develops acute infection during gestation. The risk for infection to the neonate is unrelated to the presence or absence of symptoms in the mother, but severity of infection depends on the stage of gestation at which it is acquired. Intrauterine death, microcephaly, or hydrocephaly with intracranial calcifications may develop if infection is acquired in the first half of pregnancy. Infections in the second half of pregnancy are usually asymptomatic at birth, although fever, hepatosplenomegaly, and jaundice may appear. Chorioretinitis, psychomotor retardation, and convulsive disorders may appear months or years later (Montoya & Remington, 2008). Given the risks of congenital toxoplasmosis, expectant mothers are advised against handling cat feces (e.g., cleaning the cat litter box) if the cat spends times outdoors and eating undercooked meat.

In immunosuppressed individuals, especially those with acquired immunodeficiency syndrome (AIDS), infection with *T. gondii* usually manifests with CNS involvement. Other possible clinical and pathologic manifestations include pneumonitis, myocarditis, retinitis, pancreatitis, or orchitis (McAuley & Singh, 2019). Toxoplasmosis may be difficult to diagnose clinically and is often discovered at autopsy. These infections usually result from reactivation of a latent infection acquired months or years before but occasionally result from a primary infection. Treatment is based on numerous factors, including the immune status of the host and form of infection. Treatment is indicated for congenitally infected neonates and immunocompromised patients but not typically for acute infection in immunocompetent adults. Pyrimethamine is the standard therapy along with leucovorin to protect against bone marrow suppression (CDC, 2018b; Drugs for Parasitic Infections, 2013). Management during pregnancy varies with treatment center. Spiramycin may be administered to prevent spread to the fetus or when infection is diagnosed prior to 18 weeks' gestation; however, pyrimethamine, sulfadiazine, and leucovorin are recommended when infection of the fetus is suspected or when diagnosed at or after 18 weeks' gestation (CDC, 2018b; Drugs for Parasitic Infections, 2013).

Serology remains the primary approach in establishing a diagnosis of toxoplasmosis in immunocompetent hosts (Robert-Gangneux & Dardé, 2012; Wilson & Nutman, 2015; McAuley & Singh, 2019). The Sabin-Feldman dye test and the IFA test are standards against which other methods are compared, although the former is performed in only a few centers. EIA tests are commercially available and generally provide results similar to those of the IFA. Antibodies appear in 1 to 2 weeks, and titers peak at 6 to 8 weeks. Tests for IgM-specific antibodies are especially useful for diagnosis of congenital and acute infection, but knowledge of test limitations, specifically the occurrence of false-positive reactions, is extremely important. The persistence of IgM-specific antibodies, sometimes for 1 year or longer, also is problematic and must be interpreted in conjunction with IgG antibody results. Because many persons have had asymptomatic infection, low IgG titers have little significance. Titers in patients with chronic ocular infection may also be low. Immunocompromised patients, such as those with AIDS who have active *Toxoplasma* infection, almost always have preexisting specific IgG antibodies, although titers may be low and IgM antibodies are infrequently detected. As discussed in the Serologic Diagnosis section earlier in the chapter, IgG avidity testing may be useful for differentiating between acute and remote infection. Interpretation of IgG and IgM antibody titers varies by test method and by manufacturer. The laboratory performing the test should provide the necessary interpretive criteria (McAuley & Singh, 2019).

Diagnosis of toxoplasmosis may also be established by examination of tissues, blood, or body fluids (McAuley & Singh, 2019). Demonstration of tachyzoites or tissue cysts is definitive but may prove difficult to demonstrate in H&E-stained sections; fluorescence or immunohistochemical stains, if available, are useful. Giemsa is good for staining smears of body fluids and tissue imprints. Organisms may be demonstrated by inoculating appropriate material into tissue culture or uninfected mice, although this method is not widely available. Recovery in routine viral cultures also has been described but requires extended incubation. Isolation of organisms from blood or body fluid serves as evidence of acute infection, whereas recovery from tissues may reflect chronic infection. In smears, tachyzoites are crescent-shaped or oval, measuring approximately 3 × 7 μm; cysts measure up to 30 μm in diameter and are usually spherical, except in muscle fibers, where they appear elongate (McAuley & Singh, 2019) (see Figs. 65.6H and 65.6I).

In recent years, PCR has been increasingly used to detect toxoplasmic encephalitis, disseminated disease, and intrauterine infection. Testing is available from most reference laboratories, the CDC, and select research laboratories. PCR is now an important component of testing for pregnant women, neonates, and immunocompromised hosts (McAuley & Singh, 2019).

OPPORTUNISTIC FREE-LIVING AMEBAE

Amebae of the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* are inhabitants of soil, water, and other environmental substrates, where they feed on other microscopic organisms, especially bacteria and yeasts. All three genera have been associated with opportunistic infection of the CNS, and *Acanthamoeba* spp. can also cause keratitis (Visvesvara et al., 2007; Cope et al., 2019). There has also been a single report of *Paravahlkampfia francinae* being detected in the CSF of a patient with primary amebic meningoencephalitis (PAM) (Visvesvara, et al., 2009), and *Sappinia pedata* in a left temporal lobe brain biopsy in a patient with amebic encephalitis (Cope et al., 2019; Gelman et al., 2001; Qvarnstrom et al., 2009).

PAM, caused by the ameboflagellate *Naegleria fowleri*, typically affects children and young adults who have been swimming, jumping, or diving in warm freshwater lakes or pools, or people of any age by the improper use of nasal irrigation systems. The ameboflagellate enters the brain via the cribriform plate and olfactory bulbs and reaches the frontal lobes, where it produces an acute hemorrhagic meningoencephalitis that is usually fatal within 1 week of onset of symptoms. The disease has an extremely poor prognosis, despite vigorous therapeutic intervention, including induction of therapeutic coma. Drugs that have been used with limited success include amphotericin B, rifampin, fluconazole, azithromycin, and miltefosine (Drugs for Parasitic Infections, 2013). Antemortem diagnosis is made occasionally by identifying typical trophozoites in CSF on direct wet mounts, in stained preparations (Fig. 65.8A), or in culture. However, diagnosis is usually established at autopsy examination by the finding of trophozoites (only) in tissue sections (Fig. 65.8B). Trophozoites measure 10 to 35 μm; have a large, round, central karyosome; and if exposed to warm distilled water, convert to flagellated forms in 1 to 2 hours. Cysts are not seen in clinical specimens. Culture usually is performed on nonnutrient agar plates (1.5% agar, 0.5% sodium chloride, pH 6.6–7.0) seeded with a lawn of heat-killed or living *Escherichia coli* (Garcia, 2010, 2016). Amebae ingest the bacteria, leaving tracks in the bacterial lawn, which may be seen under low-power magnification using reduced light.

Granulomatous amebic meningoencephalitis (GAE) may be caused by several species of *Acanthamoeba*, including *A. castellani*, *A. culbertsoni*, *A. polyphaga*, and *A. astronyxis*, among others (Marciano-Cabral & Cabral, 2003). It is usually a subacute or chronic opportunistic infection of chronically ill, debilitated, and immunosuppressed individuals, leading to death weeks to months following onset of symptoms. Infection is thought to spread hematogenously from primary foci in skin, the pharynx, or the respiratory tract. Systemic infections occur in individuals with AIDS and may present as ulcerative skin lesions, subcutaneous abscesses, or erythematous nodules (Cope et al., 2019). Exposure to fresh water is not necessary because cysts of *Acanthamoeba* readily become airborne and may be recovered from the throat and nasal passages (Cope et al., 2019). The pathologic reaction in tissues is necrosis with acute inflammation. Despite the name of the disease, granulomas are not commonly seen but may be present in immunocompetent hosts. Trophozoites, and less commonly cysts, are seen within brain parenchyma, skin, and, rarely, lung sections. Organisms are commonly clustered around blood vessels, reflecting their hematogenous origin. Diagnosis usually is established at autopsy, but organisms may be recognized in brain biopsies or recovered using the culture technique described for *Naegleria*. Therapeutic drugs that have been used with some success are pentamidine, sulfadiazine, flucytosine, fluconazole, and miltefosine (Drugs for Parasitic Infections, 2013).

Acanthamoeba trophozoites are somewhat larger than *Naegleria*, measuring 15 to 45 μm, and display needlelike filamentous projections from the cell known as *acanthopodia*. Cysts measure 10 to 25 μm and are double-walled, displaying a wrinkled outer wall (ectocyst) and a polygonal, stellate, or round inner wall (endocyst). Identification to the species level is problematic and reflects uncertainty as to the validity of the 18 or more described species, although it is usually not required for clinical management. Currently, genotyping is the preferred approach used in differentiating types of *Acanthamoeba* (Marciano-Cabral & Cabral, 2003).

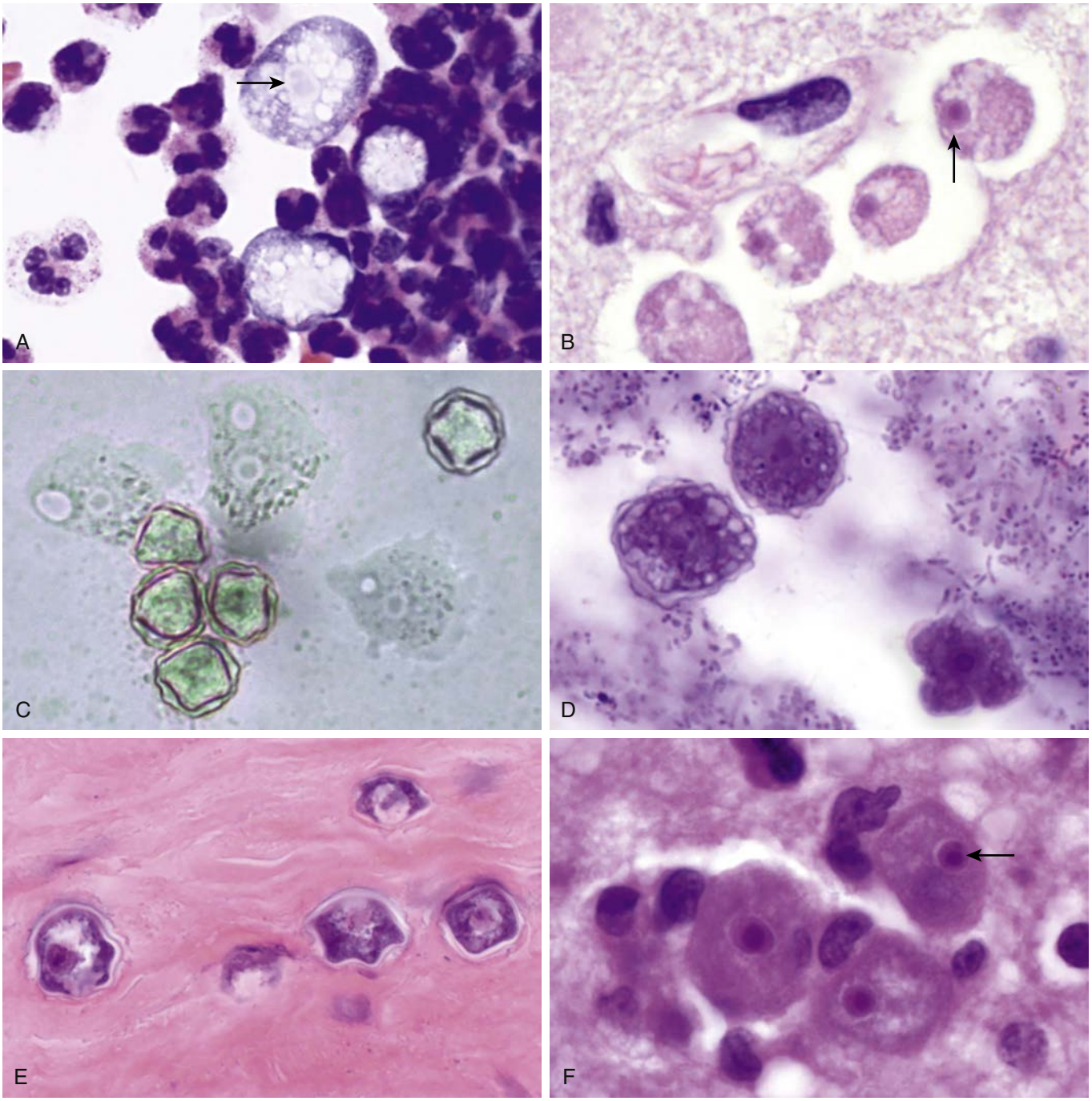


Figure 65.8 **A,** *Naegleria fowleri* trophozoites in the cerebrospinal fluid in a case of primary amebic meningoencephalitis (PAM). Note the pale nucleus with large karyosome *(arrow)* (Giemsa; 1000×). **B,** *Naegleria fowleri* trophozoites in an H&E-stained brain section in a case of PAM; note the small nucleus with large central karyosome *(arrow)* (1000×). **C,** *Acanthamoeba* sp. trophozoites and cysts on an agar culture (1000×). **D,** *Acanthamoeba* sp. cysts *(left)* and trophozoite *(right)* in contact lens fluid in a case of acanthamebic keratitis. There are abundant bacteria also present (H&E; 1000×). **E,** Double-walled cysts of *Acanthamoeba* sp. within corneal stroma (H&E; 1000×). **F,** Trophozoites in brain tissue in a case of *Balamuthia mandrillaris* granulomatous amebic encephalitis. The small nucleus with central large karyosome is highlighted *(arrow)* (H&E; 400×).

Immunofluorescence and immunoperoxidase techniques may prove useful in identifying and differentiating species and are available from the CDC (Cope et al., 2019).

GAE may also be caused by the leptomyxid ameba, *Balamuthia mandrillaris* (Cope et al., 2019). Treatment and prognosis are similar to GAE caused by *Acanthamoeba* spp. Morphologically, *Balamuthia* cannot be easily differentiated from *Acanthamoeba* by routine histology, although differences may be detected at the ultrastructural level. These organisms are antigenically distinct and may be identified using specific monoclonal or polyclonal antisera in DFA or immunoperoxidase assays (Cope et al., 2019). *Balamuthia* does not grow on agar plates used for *Naegleria* and *Acanthamoeba* but can be recovered in tissue culture using mammalian cell lines.

Acanthamoeba keratitis is an increasingly recognized painful infection of the cornea that is most likely to occur in persons who use daily-wear or extended-wear soft contact lenses or who have experienced trauma to the cornea. Incomplete or infrequent disinfection and use of homemade saline and multipurpose solutions are known risk factors for acquiring the infection (Cope et al., 2019). The disease is characterized by development of a paracentral ring infiltrate of the corneal stroma, which progresses to ulceration and possible perforation, with loss of the eye. The infection may be confused with fungal, bacterial, or herpetic keratitis but is characteristically refractory to commonly used antimicrobials. Optimal treatment varies based on the extent of disease, with topical biguanides and diamidines for uncomplicated disease (Clark et al., 2012; Drugs for Parasitic Infections, 2013). Keratoplasty is required in cases of extensive and refractory disease.

Diagnosis usually is established by demonstrating amebic trophozoites or cysts in corneal scrapings or biopsies (Fig. 65.8E). A variety of permanent stains can be used to highlight the organisms, including Giemsa, PAS, and trichrome. Use of the fluorochrome Calcofluor white is especially helpful in recognizing amebic cysts (Garcia, 2016). While cultures (described earlier, Fig. 65.8C) provide increased sensitivity over staining

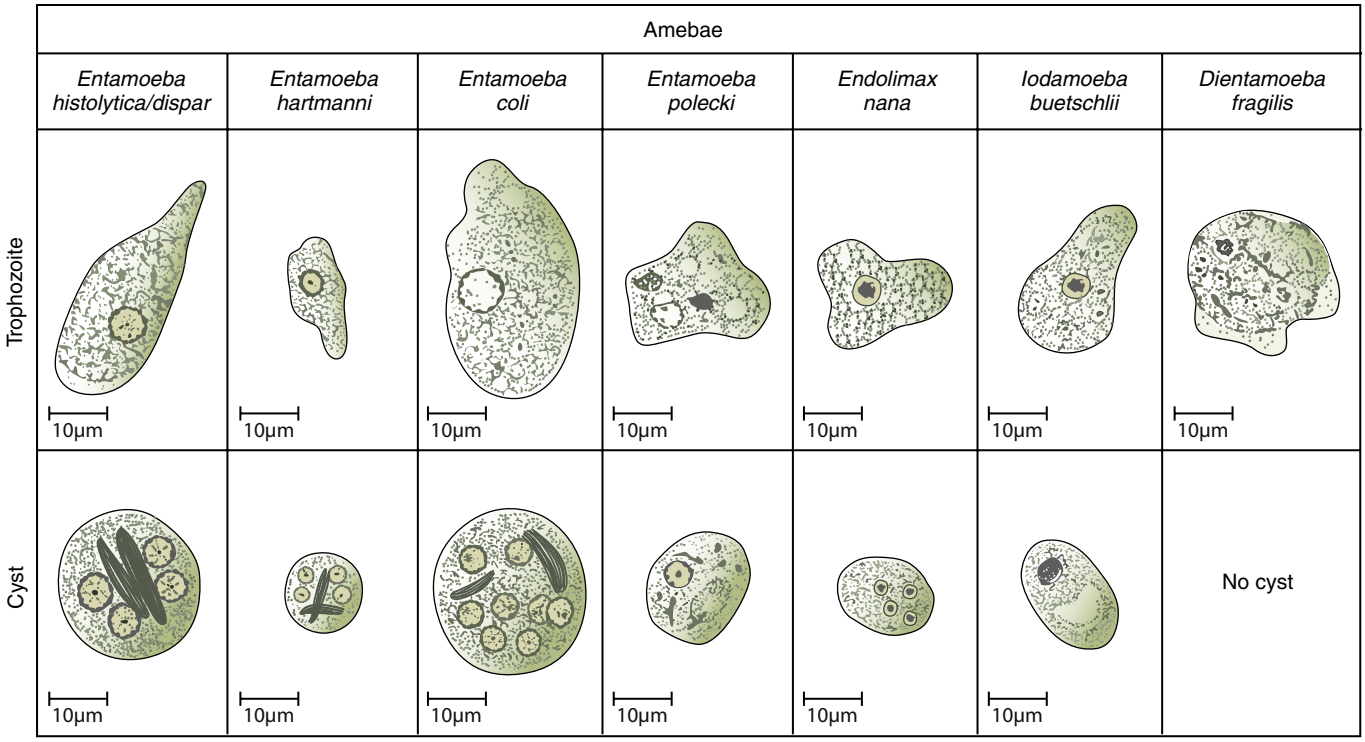


Figure 65.9 Amebae found in human stool specimens. (*Dientamoeba fragilis* is a flagellate but morphologically similar to amebae.)

methods and are often available from clinical laboratories, the sensitivity achieved by PCR may equal or exceed that of culture (Boggild et al., 2009).

INTESTINAL AND UROGENITAL PROTOZOA

Protozoa inhabiting the intestinal tract of humans include amebae, flagellates, ciliates, and coccidia, many of which are considered nonpathogens. Microsporidia also inhabit the human intestinal tract. They were historically grouped with the intestinal protozoa but are now known to be highly specialized fungi and are discussed elsewhere. Infection rates vary widely by population tested and the method employed. Most intestinal infections with protozoa are thought to be acquired by fecal-oral contamination directly from food handlers or indirectly via contaminated water.

For most laboratorians, identification of intestinal protozoa can be one of the more difficult aspects of parasitology. These organisms are small, and pathogenic species must be differentiated from nonpathogenic species and from inflammatory cells, epithelial cells, yeasts, pollen, and other confusing objects. Numerous characteristics assist in identifying intestinal protozoa. Motility patterns are classically described for many amebae, flagellates, and the ciliate *Balantioides coli,* but these are appreciated only when wet mount examination of fresh material is available. More importantly, features such as size, shape, and nuclear and cytoplasmic characteristics are used for identification in wet mounts of concentrated specimens and permanently stained preparations.

Size is an important feature (Figs. 65.9 and 65.10); thus, a properly calibrated ocular micrometer must be available for routine diagnostic use. Shape is also a helpful feature; flagellates generally are elongated and tapered, with a nucleus or nuclei at one end, whereas amebic trophozoites are rounded to oval with occasional pseudopod projections. Other important identifying morphologic features are the number and size of nuclei and the pattern of chromatin distribution, best seen in permanent stained preparations, and cytoplasmic structures such as fibrils in flagellates, ingested materials in amebic trophozoites, and glycogen masses and chromatoid bodies in amebic cysts.

During examination by any method, both nuclear and cytoplasmic characteristics should be assessed from a number of individual organisms to complete the identification. When reporting the presence of two or more species in a sample, the observer should be able to define distinct populations of organisms to prevent confusion with an occasional organism with an atypical appearance.

As mentioned in the previous section on Laboratory Methods, trophozoites typically predominate in liquid stool but degenerate within 30 minutes to 1 hour after passage unless the specimen is placed into a fixative. Cysts typically predominate in formed stool and are more resistant to degeneration. Formalin does not preserve trophozoites well; thus, parasites may be missed from formalin-based preparations unless permanent stained smears are also prepared and examined. Therefore, as mentioned previously, a complete stool examination should include examination of both a concentrated wet prep and permanently stained slide.

INTESTINAL AMEBAE

Three genera of amebae inhabit the intestinal tract of humans: *Entamoeba*, *Endolimax*, and *Iodamoeba*. Cysts are ingested and excyst in the small intestine. Resulting trophozoites proliferate by binary fission in the lumen of the colon. Both cysts and trophozoites may be passed in feces, but only mature cysts are infective. *Entamoeba histolytica* is the only ameba in which the trophozoites can invade tissues and cause clinical disease.

The genus *Entamoeba*, characterized by the presence of chromatin on the nuclear membrane, includes *E. histolytica*, the etiologic agent of amebiasis; *E. dispar*, a nonpathogenic species morphologically identical to *E. histolytica*; *E. hartmanni* and *E. coli*, two commensal species; and *Entamoeba polecki*, which is occasionally found in people who have contact with pigs or primates (Fig. 65.10) (Ash & Orihel, 2007). More recently, *E. moshkovskii* and *E. bangladeshi* have also been identified in human stool specimens and are morphologically indistinguishable from *E. histolytica*. Their potential pathogenicity is unknown at this writing. *Entamoeba gingivalis*, which does not have a known cyst stage, inhabits the oral cavity of people with poor oral hygiene (Ash & Orihel, 2007). *Entamoeba polecki* (Fig. 65.10F), *E. moshkovskii*, *E. bangladeshi*, and *E. gingivalis* are seen infrequently and are not described further. *Endolimax nana* and *I. buetschlii* are nonpathogenic species. *Dientamoeba fragilis* is recognized as a flagellate, although it lacks external flagella, and is discussed with the flagellates in the text but may be found with amebae in tables and figures because it is morphologically similar to them (Garcia, 2016).

Entamoeba histolytica (Amebiasis)

Most patients with *Entamoeba histolytica* infection are asymptomatic. However, *Entamoeba histolytica* may cause various clinical diseases, most commonly amebic dysentery, amebic colitis, and amebic liver abscesses (Meyers et al., 2013). General host defense mechanisms, previous contact with the parasite, diet, and the strain of *E. histolytica* may influence the severity of infection.

Amebic dysentery, which occurs infrequently in the United States, is an acute disease characterized by bloody diarrhea with abdominal pain and cramping. Trophozoite invasion of the intestinal mucosa occurs, producing ulceration that may lead to perforation and peritonitis. The more common form of disease seen in this country is amebic colitis, which may mimic

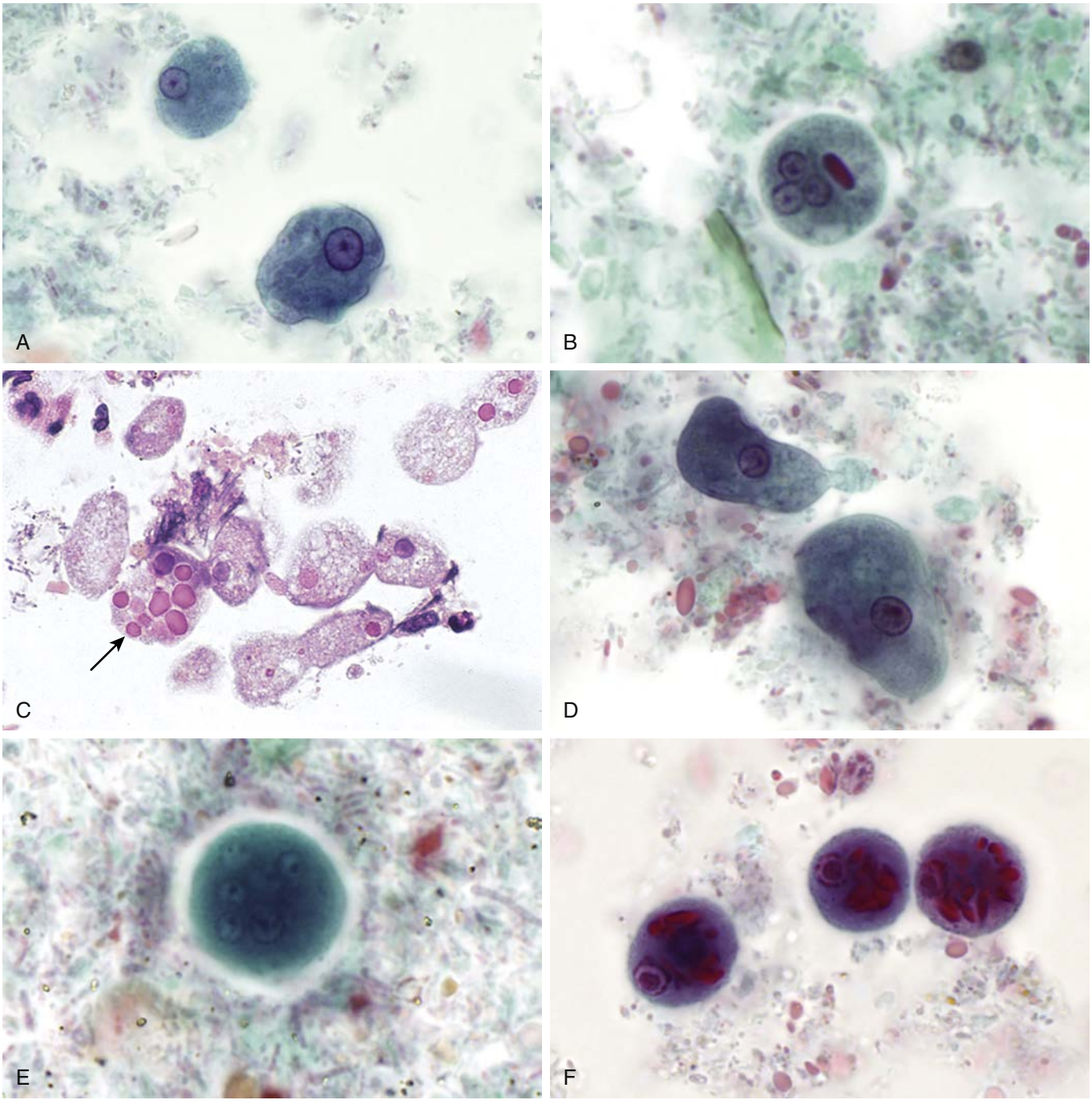


Figure 65.10 Intestinal protozoa, trichrome stain, oil immersion at 1000×, except as noted. **A,** Trophozoites of *Entamoeba histolytica/dispar*. **B,** Cyst of *E. histolytica/dispar* showing 3 of the 4 nuclei and a chromatoid bar with rounded ends. **C,** *E. histolytica* trophozoites with ingested red blood cells from a colonic lesion *(arrow)* (H&E). **D,** Trophozoites of *E. coli*. **E,** Cyst of *E. coli*. **F,** Cysts of *E. polecki* with numerous chromatoid bars.

Continued

ulcerative colitis and other forms of inflammatory bowel disease. Symptoms generally are less severe than in amebic dysentery but may include nonbloody diarrhea, constipation, abdominal cramping, and weight loss. Small, pinpoint mucosal ulcerations may develop and expand within the submucosa to form flask-shaped ulcers. All of the colon may be involved or only a portion, most commonly the cecum, rectosigmoid, or ascending colon. Less commonly, masses of granulomatous tissue, known as *amebomas*, may form in the intestine in response to the presence of amebae, producing a so-called *napkin ring lesion* that could be mistaken for a carcinoma (Meyers et al., 2013).

As *E. histolytica* trophozoites invade the intestinal wall, they may enter the bloodstream and disseminate via the portal blood supply to the liver and other organs. Amebic liver abscess (ALA) is the most common form of extraintestinal amebiasis, occurring in approximately 5% of patients with a history of intestinal amebiasis. Symptoms include fever and right upper quadrant pain. These liver abscesses are usually diagnosed by radiographic scans, ultrasound, and detection of parasite-specific host antibodies. It is important to note that *E. histolytica* cysts and/or trophozoites are present in the stool in less than half of patients at the time liver abscess is manifest; therefore, stool parasite examination is not a reliable means for diagnosis of infection. Rarely, amebic abscesses appear in other organs, such as the lung, brain, or skin, by hematogenous spread from the intestine or by contiguous spread from a liver abscess (e.g., across the diaphragm to the lungs). When the brain is involved, the trophozoites must be differentiated from those of the free-living amebae. This is usually accomplished by careful examination of the trophozoite morphology, associated host response, and clinical history. It is recommended that all patients with amebiasis be treated with suitable antiparasitic agents; asymptomatic individuals are treated with iodoquinol, paromomycin, or diloxanide furoate to eliminate the luminal stage of intestinal infection and prevent environmental shedding of infectious cysts (Drugs for Parasitic Infections, 2013). Patients with invasive or disseminated disease are first treated with metronidazole or tinidazole, both of which have good systemic penetration. This therapy should then be followed by either iodoquinol or paromomycin to eradicate any intestinal parasites (Drugs for Parasitic Infections, 2013).

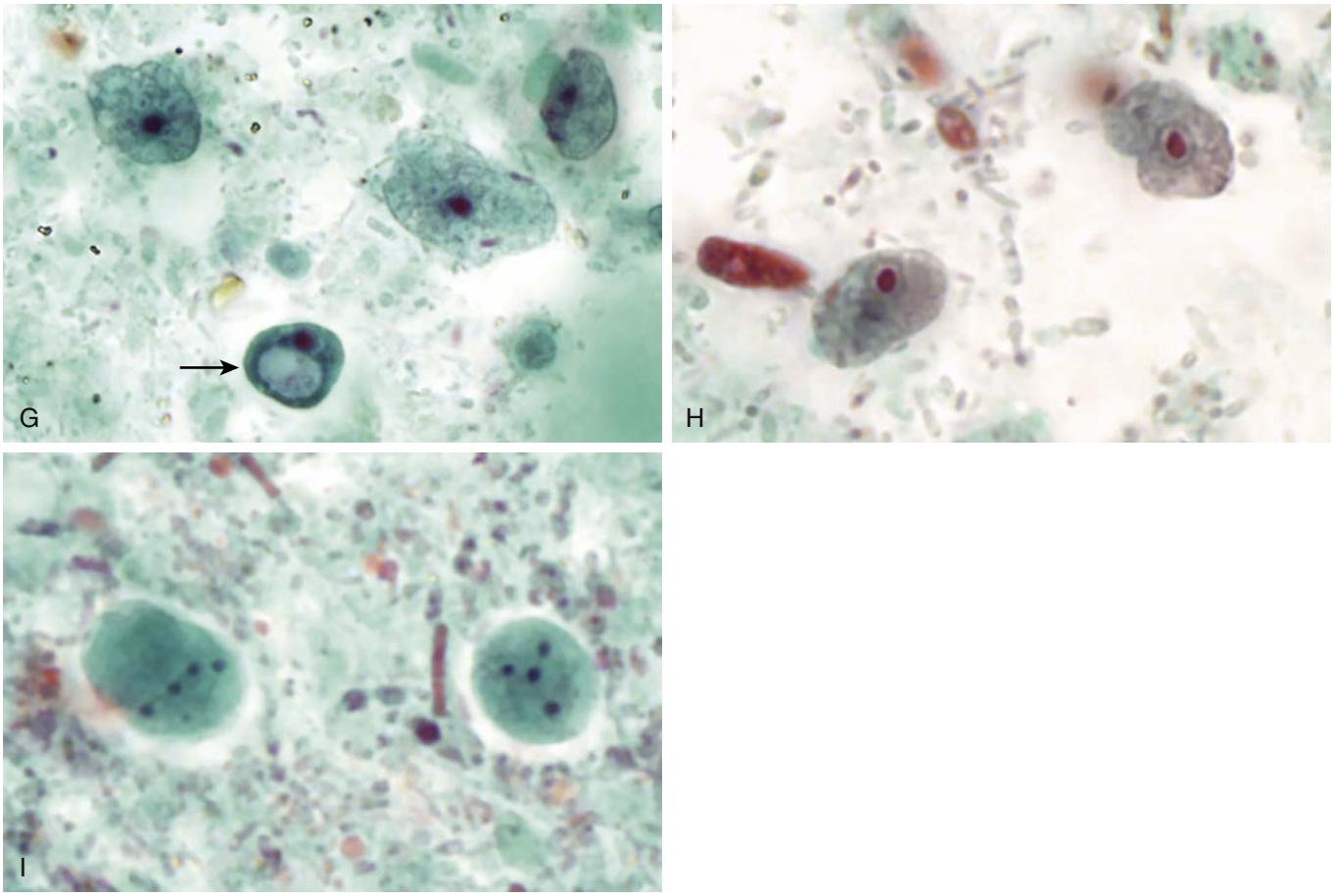


Figure 65.10—cont'd **G,** Trophozoites and cyst *(arrow)* of *Iodamoeba buetschlii*. The latter has a characteristic glycogen vacuole. **H,** Trophozoites of *Endolimax nana*. **I,** Cysts of *E. nana*.

Epidemiology

Most infections with *E. histolytica* are acquired by ingestion of contaminated food or water, although one historic outbreak was caused by a contaminated colonic irrigation machine (Istre et al., 1982). Infection may also be transmitted by oral-anal sexual activities (Pritt & Clark, 2008). Amebiasis is relatively uncommon in the United States, seen primarily in travelers and immigrants. In contrast, infection with the morphologically indistinguishable ameba, *E. dispar*, is thought to outnumber *E. histolytica* infection in the order of 9:1 (Pritt & Clark, 2008).

Diagnosis

Examination of a series of stool specimens should be sufficient for diagnosis of intestinal amebiasis in most cases, although differentiation of *E. histolytica* from *E. dispar*, *E. moshkovskii*, and *E. bangladeshi* requires additional molecular testing. The only exceptions to this are when ingested erythrocytes are seen within trophozoites in stool or trophozoites are observed in biopsy specimens or in extraintestinal sites since this is considered to be pathognomonic for invasive *E. histolytica* infection (Fig. 65.10C). If the patient has been given antibiotics or contrast media, the amebic infection may be masked for a period of time. Aspirated material from liver abscesses can be examined microscopically to detect trophozoites, although they are not commonly identifiable within the aspirated necrotic material. The last material aspirated is most likely to contain trophozoites and may be examined by direct microscopic examination or permanently stained slides. If tissue is available, sections may show organisms that stain prominently with PAS.

Culture procedures are not widely used for diagnosis but are useful for research. EIA antigen detection tests that are specific, sensitive, and able to differentiate *E. histolytica* from *E. dispar* are commercially available for stool specimens (see Table 65.4) (Garcia et al., 2018). Amplification techniques and DNA probes are also useful for differentiating *E. histolytica* from *E. dispar* (Qvarnstrom et al., 2005). At the time of this writing, there are three FDA-approved commercial molecular assays that can detect *E. histolytica*: (1) BioFire FilmArray Gastrointestinal GI Panel (BioMérieux, Marcy-l'Étoile, France), (2) BD MAX Enteric Parasite Panel (Becton Dickinson, Franklin Lakes, NJ), and (3) xTag Gastrointestinal Pathogen Panel (Luminex, Austin, TX). It is important to note that cross-reactivity with *E. dispar* may still occur with some molecular methods. The BioFire assay specifically mentions in its package insert that large numbers of *E. dispar* may result in a positive result for *E. histolytica*.

Unlike intestinal infection, ALA and other forms of disseminated disease are best detected by serologic testing. Approximately 95% of patients with ALA are seropositive; this decreases to 70% for patients with active intestinal infection and to 10% in asymptomatic carriers. Detectable titers may persist for months or years after successful treatment; as such, antibody detection cannot distinguish between past and present infection (Wilkins & Nutman, 2015).

Trophozoites of *E. histolytica* vary from 10 to 60 μm in diameter, with the nonpathogenic forms usually 15 to 20 μm and the invasive forms greater than 20 μm in greatest dimension (Table 65.8; see Figs. 65.9, 65.10A, and 65.10C) (Brooke, 1969). In direct wet mounts, trophozoites show progressive motility via rapidly formed hyaline pseudopodia that demonstrate a sharp demarcation between endoplasm and ectoplasm; unstained nuclei are not easily visible. In invasive disease, some trophozoites contain ingested erythrocytes (see Fig. 65.10C), a feature diagnostic of *E. histolytica* infection. In stained preparations, the peripheral nuclear chromatin is often evenly distributed along the nuclear membrane as fine granules. The karyosome is small and is often centrally located, with fine fibrils that generally are not visible, attaching it to the nuclear membrane. Variations in nuclear structure occur, with some karyosomes located eccentrically and peripheral chromatin irregularly distributed. As mentioned previously, the only characteristic that is pathognomonic for *E. histolytica* in stool is phagocytosis of erythrocytes. The cytoplasm is finely granular and, in cases of invasive disease, it is common to see only ingested erythrocytes. In contrast, the cytoplasm of noninvasive organisms may contain ingested bacteria or yeasts, which should not be mistaken for erythrocytes. In degenerating organisms, the cytoplasm may become vacuolated and nuclei may show abnormal chromatin clumping.

Cysts of *E. histolytica* are spherical and measure 10 to 20 μm (usually 12–15 μm) in diameter (Table 65.9; see Figs. 65.9 and 65.10B). The rounded precyst stage has a single nucleus but does not have a refractile cyst wall. As it matures, the cyst develops four nuclei, each approximately one-sixth the diameter of the cyst. Cyst nuclei appear similar to those of

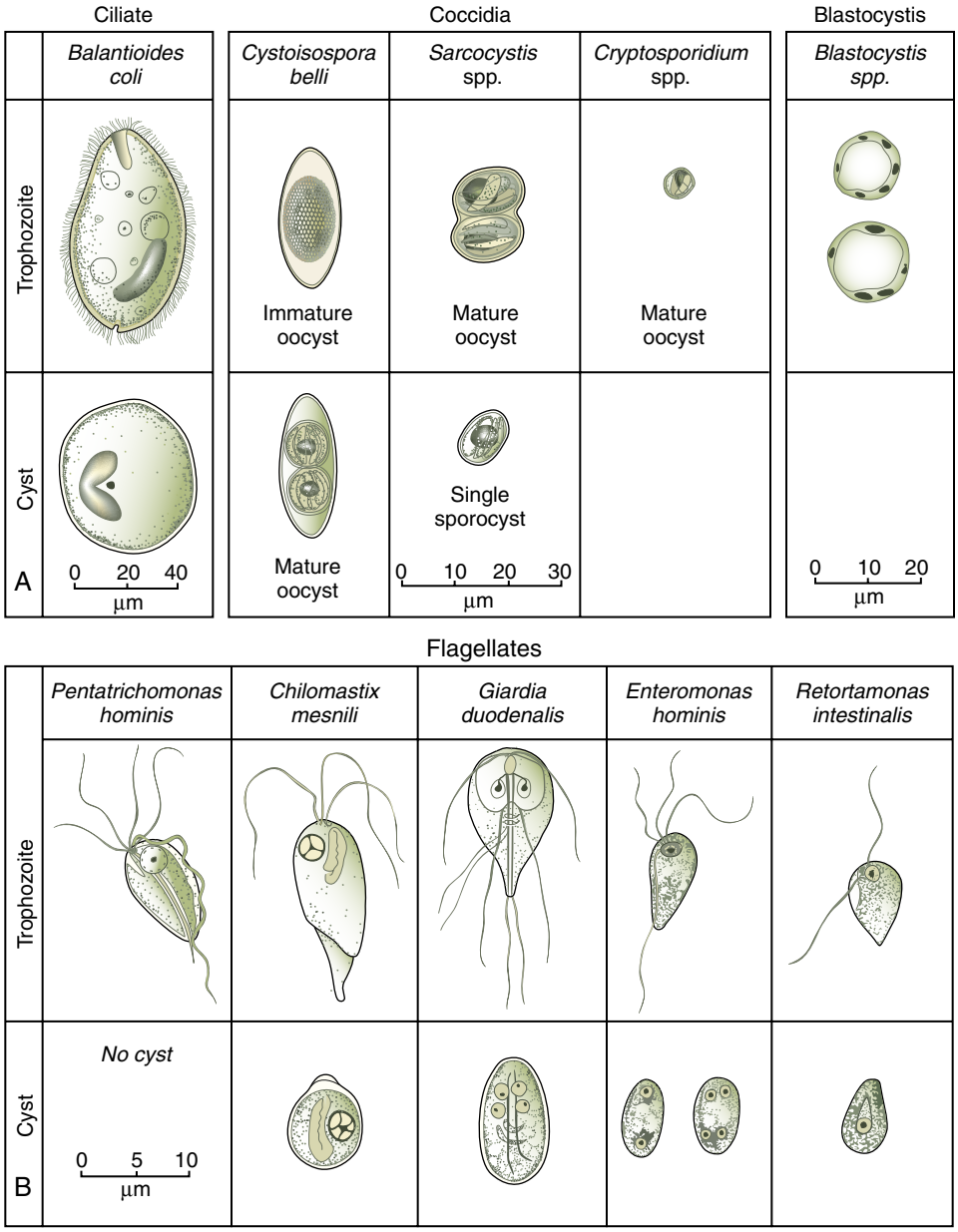


Figure 65.11 **A**, *Ciliate*, *Coccidia*, and *Blastocystis* spp. found in stool specimens of humans. **B**, Flagellates found in stool specimens of humans. (Modified from Brooke MM, Melvin DM: Morphology of diagnostic stages of intestinal parasites of man, Publication No. [CDC] 848116, Washington, DC, 1984, U.S. Department of Health and Human Services.)

trophozoites, but their smaller size makes them less useful as differentiating features. The cyst cytoplasm may contain glycogen vacuoles and chromatoid bodies with blunted or rounded ends. The number and size of nuclei and the appearance of chromatoid bodies are important diagnostic criteria for identifying cysts.

Those laboratories that do not use one of the immunologic or molecular methods to differentiate *E. histolytica* from *E. dispar* should report findings of *E. histolytica/E. dispar* or similar. A reporting comment may be added to suggest additional species-level testing if clinically indicated.

Nonpathogenic Amebae

Laboratory personnel must be able to differentiate nonpathogenic or commensal intestinal amebae from *E. histolytica/E. dispar* and *D. fragilis* (a flagellate), which are potential pathogens. Identification characteristics, best visualized in permanent stained sections, are summarized in Tables 65.8 and 65.9 and in Figures 65.9 and 65.10. Identification of trophozoites is based on size and nuclear and cytoplasmic characteristics; identification of cysts is based on size, number and characteristics of nuclei, and presence and character of chromatoid bodies and glycogen masses.

Entamoeba hartmanni has morphologic characteristics that are extremely similar to those of *E. histolytica* except that the trophozoites have a maximum diameter of 12 µm and cysts have a maximum diameter of 10 µm but are generally consistently less than 10 µm in diameter. Historically, *E. hartmanni* was called the *small race* of *E. histolytica* until it was recognized as a distinct species. Differentiation requires careful measurement of a representative sample of organisms with a properly calibrated ocular micrometer. The shrinkage of *E. histolytica/E. dispar* cysts and trophozoites in fixatives may result in an overreporting of *E. hartmanni*.

Entamoeba coli, a common lumen-dwelling ameba, may also be difficult to differentiate from *E. histolytica* given its morphologic similarities. In general, the cysts and trophozoites of *E. coli* are larger than those of *E. histolytica*, but significant size overlap exists and, therefore, size is not a reliable feature. The most definitive morphologic feature is the presence of 8 (or, rarely, 16) nuclei in mature cysts of *E. coli* compared with the usual 4 of *E. histolytica*, *E. dispar*, *and E. hartmanni*. Other helpful features are the characteristics of the cytoplasm. The cytoplasm of *E. coli* trophozoites stains somewhat more darkly than the cytoplasm of *E. histolytica* and is more vacuolated, containing numerous ingested bacteria, yeasts, and other materials. Chromatoid bodies, when present in *E. coli* cysts, are irregular in shape, with splintered or pointed ends rather than the rounded ends seen in *E. histolytica*. Although nuclear characteristics differ slightly from those of *E. histolytica* (see Figs. 65.10D and 65.10E), significant overlap may occur, especially in specimens that have not been promptly preserved. Therefore, distribution of peripheral chromatin and karyosomes should not be given great emphasis in identification of *Entamoeba* spp.

The nuclei of *I. buetschlii* trophozoites and cysts have a large, centrally located karyosome frequently surrounded by achromatic granules that may not be distinct but appear only as a muddy karyolymph space or halo. In

TABLE 65.8
Morphology of Trophozoites of Intestinal Amebae

Species	Size (in Diameter or Length)	Motility	Nucleus Number*	Peripheral Chromatin	Karyosomal Chromatin	Cytoplasm Appearance	Inclusions
Entamoeba histolytica/E. dispar	10–60 μm; usual range, 15–20 μm commensal form†; over 20 μm for invasive form‡	Progressive, with hyaline, fingerlike pseudopods	1 Not visible in unstained preparations	Fine granules; usually evenly distributed and uniform in size	Small, discrete; usually central but occasionally eccentric	Finely granular	Erythrocytes occasionally in invasive forms; noninvasive, contain bacteria
Entamoeba hartmanni	5–12 μm; usual range, 8–10 μm	Usually nonprogressive but may be progressive occasionally	1 Not visible in unstained preparations	Similar to *E. histolytica*	Small, discrete, often eccentric	Finely granular	Bacteria
Entamoeba coli	15–50 μm; usual range, 20-25 μm	Sluggish, nonprogressive with blunt pseudopods	1 Often visible in unstained preparation	Coarse granules, irregular in size and distribution	Large, discrete, usually eccentric	Coarse, often vacuolated	Bacteria, yeasts, or other materials
Endolimax nana	6–12 μm; usual range, 8–10 μm	Sluggish, usually nonprogressive with blunt pseudopods	1 Visible occasionally in unstained preparations	None	Large, irregularly shaped	Granular, vacuolated	Bacteria
Iodamoeba buetschlii	8–20 μm; usual range, 12–15 μm	Sluggish, usually nonprogressive	1 Not usually visible in unstained preparations	None	Large, usually central; surrounded by refractile, achromatic granules; these granules often are not distinct even in stained slides	Coarsely granular, vacuolated	Bacteria, yeasts, or other materials
Dientamoeba fragilis			2 (In approximately 20% of organisms, only 1 nucleus is present) Nuclei invisible in unstained preparations	None	Large cluster of 4–8 granules	Finely granular, vacuolated	Bacteria

Modified from Brooke MM, Melvin DM: *Morphology of diagnostic stages of intestinal parasites of man*, PHS Publication No. 1966, Bethesda, MD, 1969, U.S. Department of Health, Education, and Welfare.
*Visibility is for unfixed material. Nuclei sometimes may be visible in fixed material.
†Usually found in asymptomatic or chronic cases; may contain bacteria.
‡Usually found in acute cases; often contain red blood cells.

some nuclei, the halo is clear without evident achromatic granules, making the organism indistinguishable from *E. nana*. Cysts of *I. buetschlii* contain a single nucleus, in which the karyosome is often eccentric with a nearby crescent of achromatic granules (Fig. 65.10G). The cyst is characterized by a prominent vacuole of glycogen (Fig. 65.10G) that stains reddish brown in iodine-stained wet mounts. Glycogen is dissolved by aqueous fixatives and may not be demonstrable in material that has been stored.

Endolimax nana is the smallest ameba to infect humans. Trophozoites often have atypical nuclei that contain a triangular chromatin mass, a band of chromatin across the nucleus, or two discrete masses of chromatin on opposite sides of the nuclear membrane (Fig. 65.10H). A clear halo or karyolymph space surrounds the karyosome and extends to the nuclear membrane. Atypical nuclear forms may be helpful in differentiating *E. nana* from *I. buetschlii*, which is similar in appearance but larger. Cysts of *Endolimax* (Fig. 65.10I) usually contain four nuclei, although smaller numbers may be seen. Glycogen, when present, occurs diffusely in the cytoplasm rather than as a discrete mass.

BLASTOCYSTIS SPP.

Blastocystis is an enigmatic protozoan that inhabits the large bowel and is frequently found in stool specimens of asymptomatic individuals. Although appearing in stains as an ameba-like protozoan, it is now known to be a *Stramenopile* (a group that includes diatoms and algae) and is placed in the Stramenopiles-Alveolata-Rhizaria (SAR) clade (Adl et al., 2012, 2019). Traditionally, *Blastocystis* spp. have been described based on host data (*B. hominis* from humans, *B. ratti* from rats, etc.) and historically all isolates of humans were referred to as *B. hominis*. However, molecular analyses appear to demonstrate that there is no population unique to humans and all human isolates appear to have animal reservoirs. Nine (possibly 10) primary subtypes have been found in mammals and birds, with subtypes 1 and 3 making up most of the cases of human blastocystosis. It is becoming more common for clinical laboratories to report *Blastocystis* only at the genus level (Stensvold et al., 2007). Some studies have linked heavy infection to symptomatic intestinal disease, although this remains controversial (Coyle et al., 2012). Treatment options include metronidazole, trimethoprim/sulfamethoxazole, iodoquinol, and nitazoxanide (Drugs for Parasitic Infections, 2013).

Traditionally, the forms in human stool were referred to as cyst-like forms; however, those are not believed to be one of the different vaculoar forms seen in stool specimens (Fig. 65.12G). Occasionally, these forms may be seen dividing on stained slides. Because the morphologic separation of the various stages is not as clear-cut as with other protozoa, it is not common to identify and/or report the stages of *Blastocystis*. The forms in stool can vary greatly in size, from 5 to 40 μm in diameter. Characteristically, stool forms contain a large central body (similar to a vacuole) surrounded by a thin rim of cytoplasm that contains nuclei and other organelles. In a properly preserved specimen stained with trichrome, the central body is usually green while the surrounding organelles stain red (Fig. 65.12G). *Blastocystis* should be reported and quantified when present, especially when they are numerous (five or more per 1000× field), since quantity may correlate with clinical presentation (Garcia, 2016). This is the only protozoan parasite (and the only intestinal parasite in the United States) in which the quantity is routinely reported.

FLAGELLATES

Dientamoeba fragilis

Dientamoeba fragilis is an ameboid-appearing protozoan that infects the colon and has been associated with diarrheal disease, especially in young

TABLE 65.9
Morphology of Cysts of Intestinal Amebae

Species	Size	Shape	Nucleus Number	Peripheral Chromatin	Karyosomal Chromatin	Cytoplasm Chromatoid Bodies	Glycogen
Entamoeba histolytica/*E. dispar*	10–20 μm; usual range, 12–15 μm	Usually spherical	4 in mature cyst; immature cysts with 1 or 2 occasionally seen	Peripheral chromatin present; fine, uniform granules, evenly distributed	Small, discrete, usually central	Present; elongate bars with bluntly rounded ends	Usually diffuse; concentrated mass often present in young cysts; stains reddish brown with iodine
Entamoeba hartmanni	5–10 μm; usual range, 6–8 μm	Usually spherical	4 in mature cyst; Immature cysts with 1 or 2 often seen	Similar to *E. histolytica*	Similar to *E. histolytica*	Present; elongate bars with bluntly rounded ends	Similar to *E. histolytica*
Entamoeba coli	10–35 μm; usual range, 15–25 μm	Usually spherical; occasionally oval, triangular, or of another shape	8 in mature cyst; occasionally supernucleate cysts with 16 or more are seen; immature cysts with 2 or more occasionally seen	Peripheral chromatin present; coarse granules irregular in size and distribution, but often appear more uniform than in trophozoites	Large, discrete, usually eccentric, but occasionally central	Present; usually splinterlike with pointed ends	Usually diffuse, but occasionally well-defined mass in immature cysts; stains reddish brown with iodine
Endolimax nana	5–10 μm; usual range, 6–8 μm	Spherical, ovoid, or ellipsoidal	4 in mature cysts; immature cysts with fewer than 4 rarely seen	None	Large, usually centrally located	Occasionally, granules or small oval masses seen, but bodies as seen in *Entamoeba* spp. are not present	Usually diffuse; concentrated mass seen occasionally in young cysts; stains reddish brown with iodine
Iodamoeba buetschlii	5–20 μm; usual range, 10–12 μm	Ovoid, ellipsoidal, triangular, or of another shape	1 in mature cyst	None	Large, usually eccentric; refractile, achromatic granules on one side of karyosome	Granules occasionally present, but bodies as seen in *Entamoeba* spp. are not present	Compact, well-defined mass; stains dark brown with iodine

Modified from Brooke MM, Melvin DM: *Morphology of diagnostic stages of intestinal parasites of man*, PHS Publication No. 1966, Bethesda, MD, 1969, U.S. Department of Health, Education, and Welfare.

children (Stark et al., 2016). Although similar in appearance to amebae, *Dientamoeba* is considered a flagellate on the basis of ultrastructural details and antigenic and molecular similarities. Because of the similarity of *Dientamoeba* to amebae at the light microscope level, this species has traditionally been included in figures and diagnostic algorithms for amebae (Figs. 65.11 and 65.12 *F*).

Symptoms of *D. fragilis* infection include diarrhea and abdominal distention. Recent evidence suggests that dientamoebiasis is a more frequent cause of diarrhea than previously thought: 4.3% of patients in one study harbored this organism (Stark et al. 2016). Approximately 25% of persons infected with this parasite have been reported to have symptomatic disease; however, the exact role of *D. fragilis* in symptom causation is uncertain. Treatment options include paromomycin, iodoquinol, or metronidazole (Drugs for Parasitic Infections, 2013). *Dientamoeba fragilis* infection may be overlooked unless permanently stained slides are examined. Multiple specimens may need to be submitted because shedding varies from day to day. Commonly, organisms contain two nuclei that consist of a cluster of four to eight karyosomal granules, which may appear as one large irregular karyosome (see Fig. 65.12F). Uninucleate *D. fragilis* may be confused with trophozoites of *E. nana* or *I. buetschlii*. The cytoplasm is finely granular and often contains ingested bacteria and yeast. Classically, *D. fragilis* was not thought to have a cyst stage. Cyst forms have recently been described (Stark et al., 2014), although this has not gained wide acceptance in the protozoology community. Molecular phylogenetic analyses place *Dientamoeba fragilis* within the clade Trichomonadida, none of which are known to be cyst producers (Adl et al., 2018; Adl & Mathison, 2019).

Giardia duodenalis

Giardia duodenalis, historically known as *G. intestinalis* or *G. lamblia*, is a pathogenic intestinal protozoan that causes both endemic and epidemic disease worldwide. In the United States, it is especially problematic for travelers, campers, children attending day care, and homosexual men. It frequently causes disease in individuals drinking contaminated water, and a number of large water-borne outbreaks have been described (Cama & Mathison, 2015). Pathogenic protozoa are not always killed by the usual concentrations of chlorine in municipal water supplies. Therefore, unless the water supply is filtered, it may serve as a source of infection, as it did in the Rome, New York, outbreak (Craun, 1986).

Giardia duodenalis trophozoites multiply in the small bowel and attach to the mucosa by a ventral concave sucking disk. Infection may be asymptomatic or may cause disease ranging from mild diarrhea with vague abdominal complaints to a malabsorption syndrome with diarrhea and steatorrhea, similar to that of celiac disease (also known as *celiac sprue* and *gluten-sensitive enteropathy*). The pathogenesis is not fully understood, although disruption of the integrity of the brush border with resulting disaccharidase deficiency may occur from direct or indirect effects of the organism's presence (Meyers et al., 2013). Giardiasis should be considered in any patient presenting with diarrhea of longer than 10 days' duration. The drugs of choice for giardiasis are tinidazole, metronidazole, or nitazoxanide (Drugs for Parasitic Infections, 2013).

Diagnosis is commonly established by demonstration of *Giardia* trophozoites or cysts, or both, in fecal specimens, although antigen tests and NAATs provide increased sensitivity (see following discussion). Trophozoites predominate in diarrheic stool, whereas infectious cysts are more likely to be found in formed stool. The passage of organisms varies from day to day; therefore, examination of multiple specimens, collected on different days, may be necessary. Direct wet mounts may demonstrate the characteristic "falling leaf" motility of trophozoites in diarrheic or duodenal aspirate specimens. Both cysts and trophozoites can be seen in wet mounts and permanently stained slides. Historically, small bowel aspirates or so-called string tests (Enterotest) were used to provide increased sensitivity but are uncommonly used today.

Many antigen detection methods based on DFA or EIA are commercially available (see Table 65.4) and provide increased sensitivity for detection of *G. duodenalis* in stool specimens over routine O&P testing (Cama & Mathison, 2015). Antigen detection methods may be particularly useful for patients with diarrhea who have not traveled outside of the United States since they may be used in place of the labor-intensive and subjective O&P examination, often in combination with an antigen test for *Cryptosporidium*

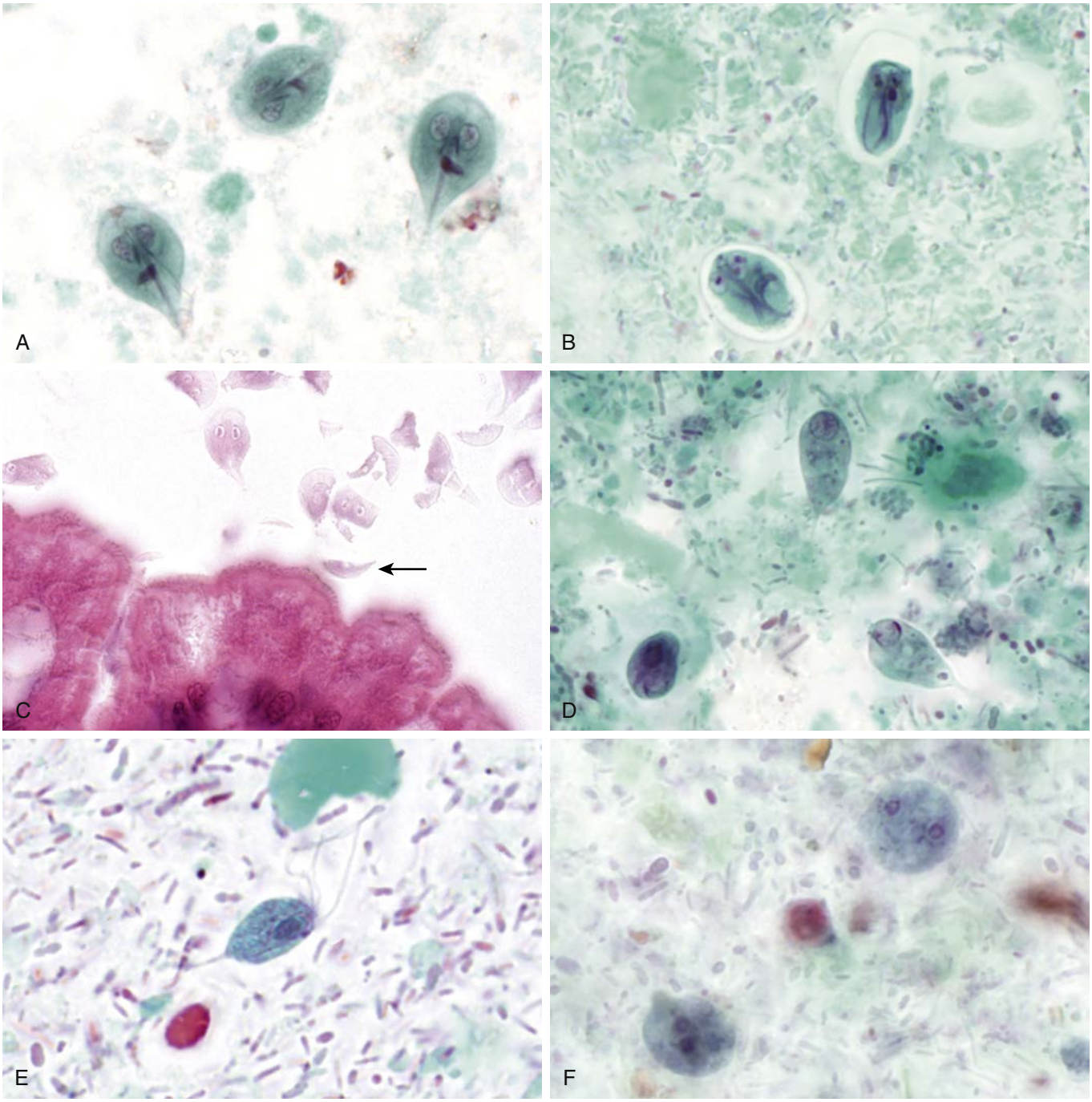


Figure 65.12 Intestinal protozoa, trichrome stain, oil immersion at 1000×, except as noted. **A,** Trophozoites of *Giardia duodenalis*. **B,** Cysts of *G. duodenalis*. **C,** Duodenal biopsy demonstrating multiple *G. duodenalis* trophozoites seen ventrally and laterally *(arrow)* (H&E). **D,** A lemon-shaped cyst of *Chilomastix mesnili (bottom left)* with visible nucleus and hyaline cap, and 2 *C. mesnili* trophozoites *(right)*. **E,** Trophozoite of *Pentatrichomonas hominis*. **F,** Trophozoites of *Dientamoeba fragilis*.

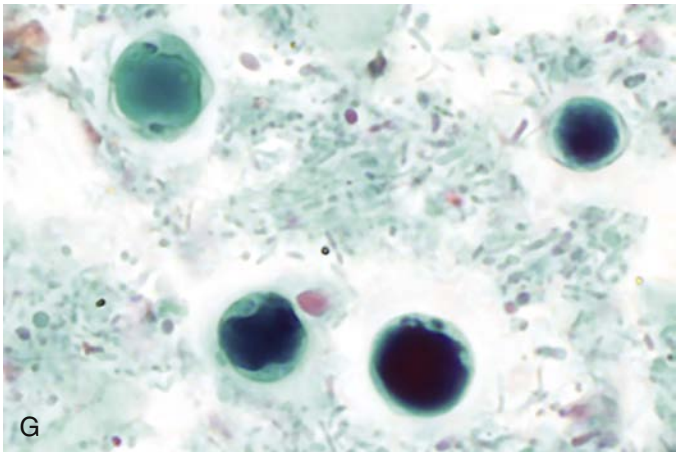
spp. However, they cannot replace the need for traditional morphologic examination of the specimen if other pathogenic intestinal parasites such as helminths are suspected. Also, use of an antigen detection method does not preclude the need for testing multiple separate stool specimens for sensitive detection of *G. duodenalis* (Hanson & Cartwright, 2001).

At the time of this writing, there are three FDA-approved multiplex NAATs that can detect *G. duodenalis* (see earlier Amebiasis section). All are components of multiplex NAATs that detect multiple bacterial, viral, and parasitic causes of diarrhea.

Giardia trophozoites are pear-shaped with a tapered posterior end, and possess a large sucking disc, two nuclei, and curved median bodies that give the appearance of a smiling face with two prominent eyes (Table 65.10; see Figs. 65.11, 65.12A, and 65.12C). When viewed from the side, the anterior end of the organism is thicker and tapers posteriorly; the anterior half to three-quarters consists of the sucking disk on the ventral surface (Fig. 65.12C). The four lateral, two ventral, and two caudal flagella are not usually evident in wet mounts or in stained preparations but are highlighted well using Giemsa-based stains. Cysts are oval and usually quadrinucleate when mature. Below the nuclei are dark-staining curved median bodies that cross the longitudinal fibrils (called *axonemes*), providing the appearance of a smiling mouth. The cytoplasm is sometimes retracted from the cyst wall.

Chilomastix mesnili

Chilomastix mesnili (see Table 65.10, Figs. 65.11, 65.12D, and 65.12E) is a nonpathogenic lumen-dwelling flagellate of humans that must be differentiated from trophozoites of amebae and *Giardia* in stained smears. The consistent location of the single nucleus at one end of the organism and the tapering of the end opposite the nucleus are helpful identifying features. If multiple organisms are examined, the cytostome and the spiral groove are commonly visible in some. The three external flagella usually are not visible in stained or formalin-fixed preparations. The lemon-shaped cysts contain a single nucleus and various curved cytostomal fibers with a safety pin–like appearance.



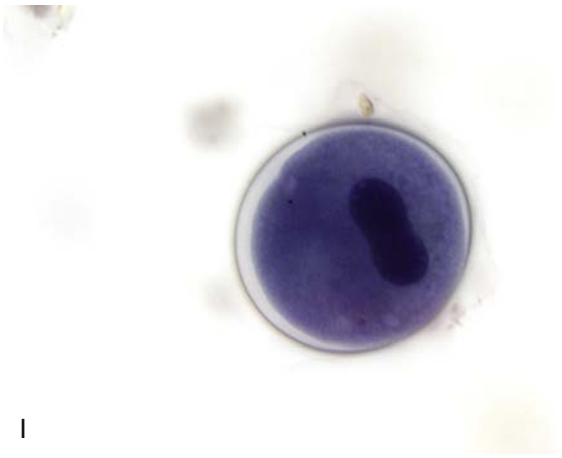


Figure 65.12—cont'd **G,** Multiple vacuolar forms of *Blastocystis* spp. **H,** Trophozoite of *Balantioides coli* in wet mount; note cilia covering the cell and the prominent macronucleus. **I,** Cyst of *B. coli* demonstrating a characteristic kidney-bean-shaped macronucleus (iron hematoxylin).

Pentatrichomonas hominis

Pentatrichomonas hominis, known previously as *Trichomonas hominis* (see Table 65.10 and Figs. 65.11 and 65.12E), is an infrequently seen, nonpathogenic intestinal flagellate that may be confused with *E. hartmanni*, small *E. histolytica* trophozoites, *E. nana*, *C. mesnili*, or *G. duodenalis*. Organisms do not stain particularly well and often are distorted in permanent smears. Several organisms may have to be examined in stained preparations to demonstrate the single nucleus, undulating membrane and associated costa, and flagella. A prominent rod-like object, the axostyle, runs through the organism and protrudes from the posterior end. No cyst stage has been described.

Trichomonas vaginalis

Trichomonas vaginalis is a common cause of vaginitis, characterized by inflammation, itching, vaginal discharge, and, occasionally, dysuria. The infection usually is spread by sexual intercourse, often to females from an asymptomatic male partner. Occasionally, males may have symptomatic prostatitis or urethritis. Trichomoniasis is the most common curable sexually transmitted infection (STI), estimated to infected 3.7 million individuals in the United States (CDC, 2018b). It is one of the five NPIs targeted by the United States for public health action. Complications of infection include preterm delivery and low birth weight in pregnant women, and increased risk of acquiring and spreading other STIs (CDC, 2018b). The drug of choice for trichomoniasis is tinidazole or metronidazole (Drugs for Parasitic Infections, 2013). Sexual partners should be simultaneously treated, regardless of symptoms.

Trichomonas vaginalis infections may be diagnosed in the physician's office by direct wet mount examination of vaginal fluid, prostatic fluid, or sediments of freshly passed urine. Morphologically, *T. vaginalis* resembles *P. hominis* but is larger (up to 23 μm), and the undulating membrane extends only half the length of the body. Because of the difference in habitat, it generally is not necessary to differentiate these trichomonads morphologically.

Despite its ease of use, direct wet mount examination is an insensitive method for detecting trichomoniasis (51%–65% sensitivity). Thus, NAATs are now the recommended method for detection (Workowski & Bolan, 2015; Meites et al., 2015). There are multiple FDA-cleared methods for detecting trichomoniasis, with sensitivities and specificities exceeding 95% (APHL, 2016; Nye, 2009). Most are cleared only for female specimens; the Xpert TV (Cepheid, Sunnyvale, CA), is the only NAAT that has FDA clearance for detection of *T. vaginalis* in both male and female specimens (Schwebke et al., 2018).

Other methods of testing include culture, antigen detection, and microscopic examination of stained smears (e.g., Papanicolaou, Giemsa) (APHL, 2016; Nye, 2009). Cultures, including use of a convenient commercial "pouch" system (In-Pouch TV, Biomed, White City, OR), were previously considered the gold standard prior to introduction of NAATs and reported sensitivities of 75% to 96% (APHL, 2016; Nye, 2009). The FDA-cleared nucleic acid probe test, BD Affirm VP III microbial identification system, also provides reasonable detection sensitivity (89.2%–92.8%) (APHL, 2016). Antigen tests generally have lower sensitivity than nucleic acid detection methods for trichomoniasis; the FDA-cleared OSOM Trichomonas Test (Sekisui Diagnostics, Burlington, MA) is a CLIA-waived EIA with reported sensitivities of 75% to 96% (APHL, 2016). Finally, Papanicolaou-stained gynecologic smears may reveal *T. vaginalis* on occasion but have poor sensitivity and specificity.

Other Flagellates

Enteromonas hominis and *Retortamonas intestinalis* are small, nonpathogenic intestinal flagellates that are seen infrequently but, when present, may occur in large numbers. Morphologic characteristics are reviewed in Table 65.10 (see also Fig. 65.11). *Trichomonas tenax*, a trichomonad that normally lives in the oral cavity of humans, has been isolated from the pleural fluid in patients with bacterial empyema. However, their presence is believed to be secondary to the bacteria, which is their normal food source (Leterrier et al., 2012).

CILIATES

Balantioides coli

The ciliate *Balantioides coli* (formerly *Balantidium coli*, syn. *Neobalantidium coli*; see Figs. 65.11, 65.12H, and 65.12I) may cause a dysentery-like syndrome with colonic ulcerations similar to that of amebiasis but only rarely disseminates outside of the intestine. Treatment is with tetracycline,

TABLE 65.10
Morphology of Intestinal Flagellates

Species	Size (Length)	Shape	Motility	Number of Nuclei	Number of Flagella*	Other Features
Trophozoites						
Pentatrichomonas hominis†	8–20 μm; usual range, 11–12 μm	Pear-shaped	Rapid, jerking	1 Not visible in unstained mounts	3–5 anterior; 1 posterior	Undulating membrane extending length of body
Chilomastix mesnili	6–24 μm; usual range, 10–15 μm	Pear-shaped	Stiff, rotary	1 Not visible in unstained mounts	3 anterior; 1 in cytostome	Prominent cytostome extending 1/3–1/2; spiral groove across ventral surface
Giardia duodenalis	10–20 μm; usual range, 12–15 μm	Pear-shaped	Falling leaf	2 Not visible in unstained mounts	4 lateral; 2 ventral; 3 caudal	Sucking disk occupying 1/2–3/4 of ventral surface
Enteromonas hominis	4–10 μm; usual range, 8–9 μm	Oval	Jerking	1 Not visible in unstained mounts	3 anterior; 1 posterior	One side of body flattened; posterior flagellum extending free, posteriorly or laterally
Retortamonas intestinalis	4–9 μm; usual range, 6–7 μm	Pear-shaped or oval	Jerking	1 Not visible in unstained mounts	1 anterior; 1 posterior	Prominent cytostome extending approximately 1/2 length of body
Dientamoeba fragilis†	5–15 μm; usual range, 9–12 μm	ameboid	Pseudopods are angular, serrated, or broad-lobed, and hyaline is almost transparent	1–2; typically binucleate forms predominate; not visible in unstained preparation	None	Cytoplasm may contain bacteria and yeast

Species	Size	Shape	Number of Nuclei	Other Features
Cysts				
Chilomastix mesnili	6–10 μm; usual range, 8–9 μm	Lemon-shaped, with anterior hyaline knob or "nipple"	1 Not visible in unstained preparations	Cytostome with supporting fibrils. Usually visible in stained preparations.
Giardia duodenalis	8–13 μm; usual range, 11–12 μm	Oval or ellipsoidal	Usually 4; not distinct in unstained preparations; usually located at one end	Fibrils or flagella longitudinally in cyst. Cytoplasm often retracts from a portion of cell wall.
Enteromonas hominis	4–10 μm; usual range, 6–8 μm	Elongate or oval	1–4, usually 2 lying at opposite ends of cyst; not visible in unstained mounts	Resembles *E. nana* cyst. Fibrils or flagella usually are not seen.
Retortamonas intestinalis	4–9 μm; usual range, 4–7 μm	Pear-shaped or slightly lemon-shaped	1 Not visible in unstained mounts	Resembles *Chilomastix* cyst. Shadow outline of cytostome with supporting fibrils extends above nucleus.

Modified from Brooke MM, Melvin DM: *Morphology of diagnostic stages of intestinal parasites of man*, PHS Publication No. 1966, Bethesda, MD, 1969, U.S. Department of Health, Education, and Welfare.
*Not a practical feature for identification of species in routine fecal examinations.
†*Pentatrichomonas* hominis and *Dientamoeba fragilis* do not have a cyst form (see text for the latter).

metronidazole, or iodoquinol (Drugs for Parasitic Infections, 2013). Human infection, rare in the United States, is usually acquired from hogs, rodents, or nonhuman primates, which are commonly infected. *B. coli* is the largest protozoan and only ciliate to infect humans. Trophozoites are between 40 μm and more than 200 μm in greatest dimension (most measure 50 to 100 μm) and are uniformly covered with cilia that are slightly longer at the anterior end adjacent to the cytostome. A large macronucleus is readily seen in stained preparations, and a smaller micronucleus is infrequently visible. Numerous food vacuoles and contractile vacuoles are present in the cytoplasm. Cysts are rounded, measuring 50 to 70 μm in length. Cilia may be seen within younger cysts, and nuclear characteristics are similar to those of trophozoites (Ash & Orihel, 2007). Stool specimens that have been contaminated with stagnant water may contain free-living ciliates, which usually can be distinguished from *B. coli* by differences in their ciliary pattern. Ciliocytophthoria in respiratory specimens has resulted in incorrect presumptive identifications of *B. coli* (Hadziyannis et al., 2000; Pritt, 2018).

Cryptosporidium and the Coccidia

Cryptosporidium and the coccidia comprise a large group of apicomplexan parasites that have a sexual stage in the intestinal tract of invertebrate and vertebrate animals (Lindsay & Weiss, 2019; Xiao & Cama, 2019). Some species also develop asexually in extraintestinal sites in host tissues. Genera infecting the intestine of humans—such as *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, and *Sarcocystis*—generally produce self-limited diarrheal disease in immunocompetent persons. Severe protracted diarrhea may develop in immunocompromised hosts following infection with *Cryptosporidium*, *Cyclospora*, and *Cystoisospora*. It should be noted that *Cryptosporidium* is no longer considered part of the Coccidia proper but is still grouped with the coccidians in the clinical realm for convenience (Adl et al., 2012, 2018; Xiao & Cama, 2019).

Cryptosporidium spp.

Cryptosporidium spp. use a single host in their life cycle. The two species most commonly implicated in human disease are *C. hominis* (a human parasite) and *C. parvum* (a livestock and ruminant parasite) (Cama & Mathison, 2015; Xiao & Cama, 2019). Parasites develop in the brush border of epithelial cells of the small and large intestine and occasionally spread to other sites, such as the gallbladder, pancreas, and respiratory tract, mainly in immunocompromised patients (Figs. 65.13A and 65.13E).

The epidemiology of cryptosporidiosis is similar to that of giardiasis. One of the largest known outbreaks of water-transmitted infection

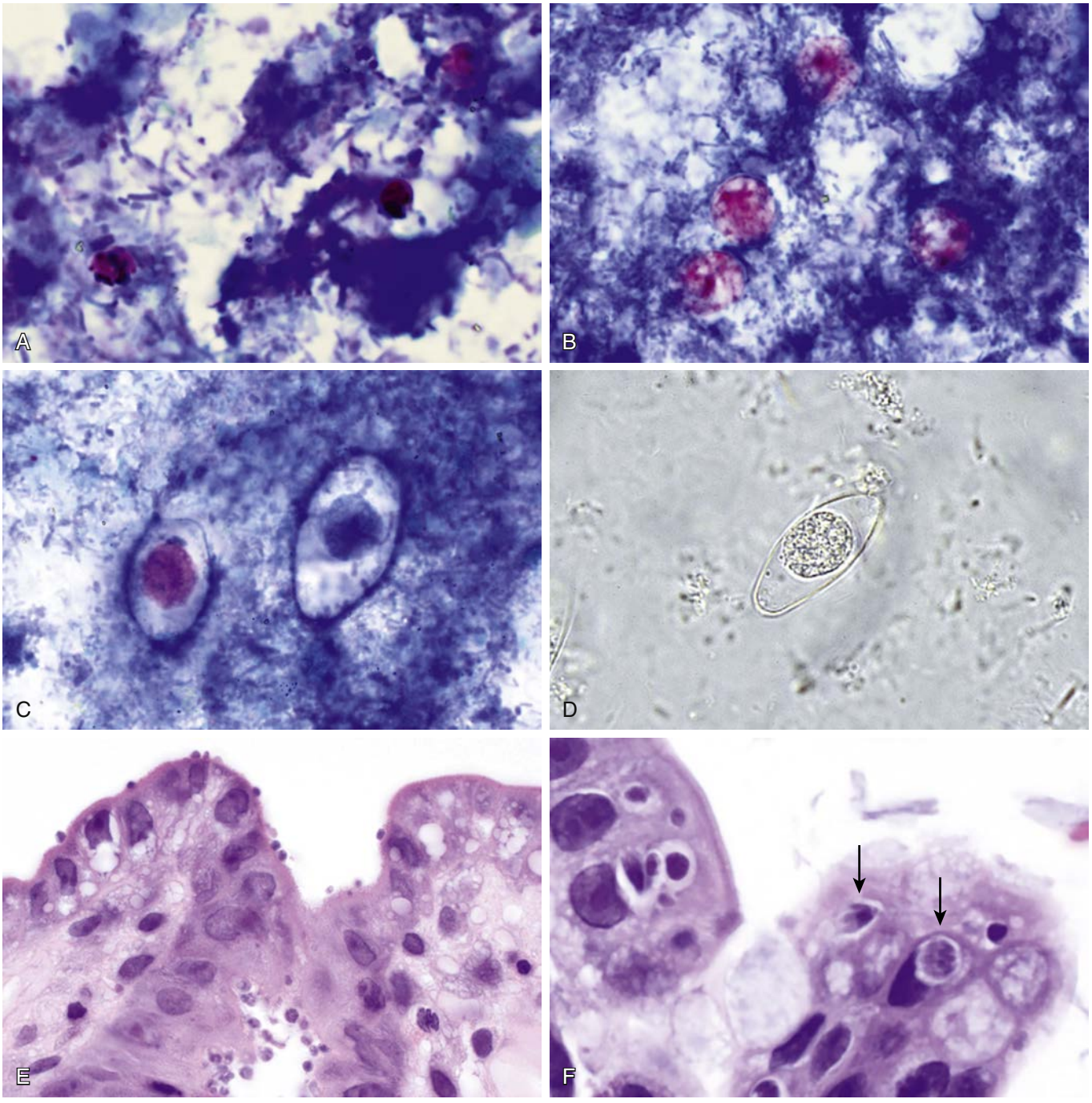


Figure 65.13 **A,** Modified acid-fast stain of a fecal smear demonstrating oocysts of *Cryptosporidium* sp. (1000×). **B,** Modified acid-fast stain of a fecal smear demonstrating oocysts of *Cyclospora cayetanensis* (1000×). **C,** Modified acid-fast stain of a fecal smear demonstrating two oocysts of *Cystoisospora belli*. Note that one is not stained, as is common with the oocysts of *C. cayetanensis* and *C. belli* (1000×). **D,** Oocyst of *C. belli* in feces (wet mount; 1000×). **E,** Small-bowel biopsy demonstrating development of *Cryptosporidium* sp. parasites within the brush border of enterocytes (H&E; 1000×). **F,** Small-bowel biopsy demonstrating development of *C. cayetanensis* parasites within enterocytes, including meronts *(arrows)* (H&E; 1000×).

Continued

occurred in Milwaukee, Wisconsin, in 1993. In that outbreak, an estimated 400,000 individuals became ill from tap water contaminated with farm runoff following heavy rains (MacKenzie et al., 1995). Similar to cysts of *Giardia*, *Cryptosporidium* oocysts are refractory to usual chlorination levels of drinking water; unless a community's water supply from a surface source is filtered, epidemics may occur.

Cryptosporidium is a common cause of acute, self-limited diarrhea in immunocompetent individuals, especially in children who attend day care. In patients with AIDS, *Cryptosporidium* may cause chronic secretory diarrhea that can last for months to years and may contribute to death. The incubation period is about 8 days; in previously healthy persons, the illness lasts 9 to 23 days. Patients may have malaise, fever, anorexia, abdominal cramps, and diarrhea (Xiao & Cama, 2019). Nitazoxanide is used to treat immunocompetent individuals, but no drug has proven efficacy in immunocompromised individuals (Drugs for Parasitic Infections, 2013); restoration of immunity, when possible, is the treatment of choice in the latter population.

Diagnosis can be made by stool examination, antigen detection, or molecular testing (see Tables 65.4 and 65.6). Various concentration methods, including formalin–ethyl acetate sedimentation and Sheather's sugar flotation, work well (Garcia, 2016). The availability of the formalin–ethyl acetate method makes this technique attractive, although centrifugation speed and times must be increased to maximize recovery (Garcia, 2010, 2016). A smear is prepared from the sediment and stained with an acid-fast stain or immunofluorescent reagents. Several acid-fast staining methods, including auramine-O, have been evaluated, but a modified cold Kinyoun method is used most widely. Spherical oocysts measure 4 to 6 μm in diameter and, when stained by the modified Kinyoun procedure, appear a deep fuchsia. However, some unevenness of staining intensity may occur, along with variability in the percentage of cysts that stain positive. Sporozoites may be visualized in the stained oocysts.

Commercial DFA and EIA reagents, which provide good sensitivity and specificity (Cama & Mathison, 2015), are especially good for laboratories where *Cryptosporidium* is infrequently encountered and where there is

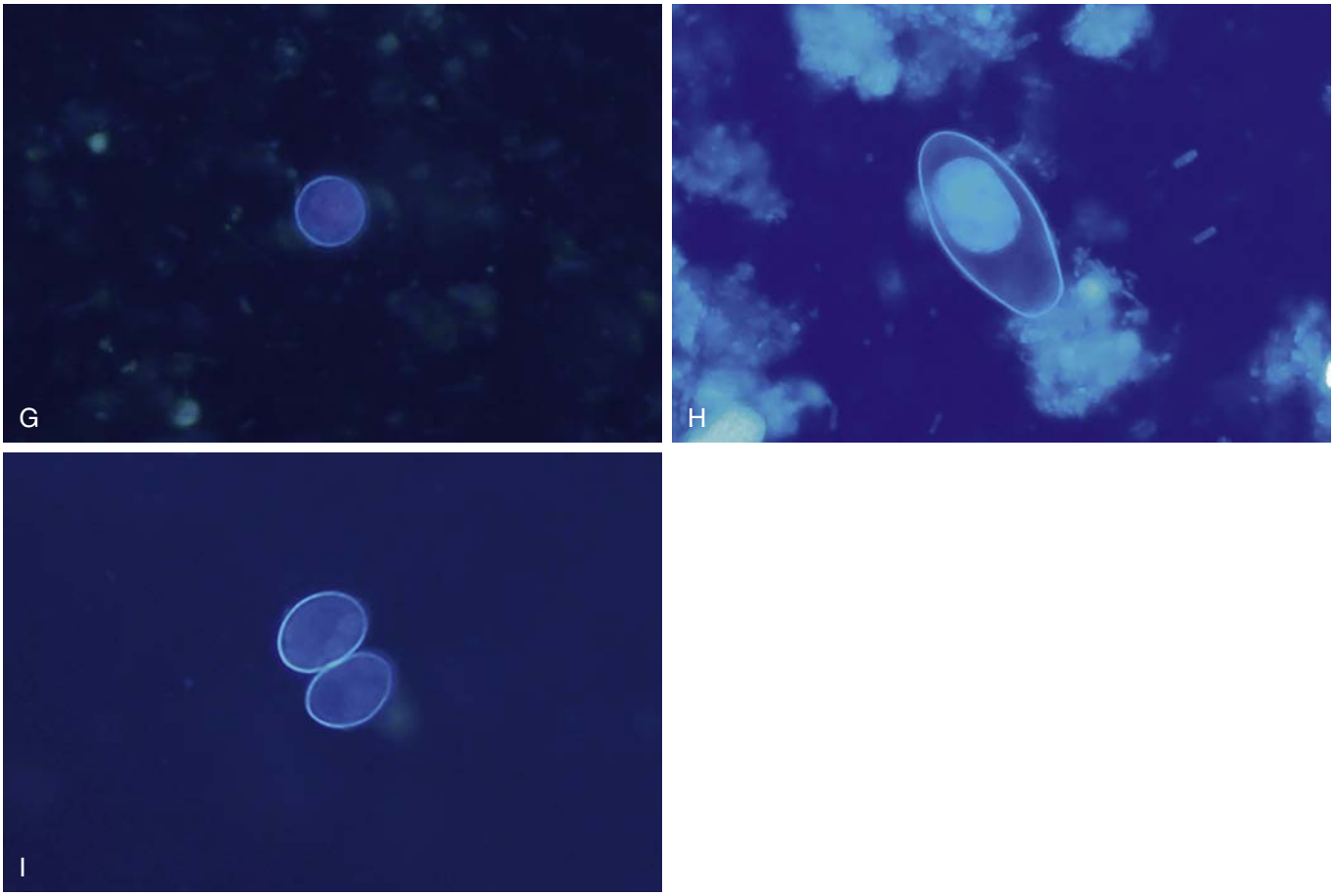


Figure 65.13—cont'd **G,** Oocyst of *C. cayetanensis* demonstrating autofluorescence (400×). **H,** Oocyst of *C. belli* demonstrating autofluorescence (400×). **I,** Oocyst of *Sarcocystis* spp. demonstrating autofluorescence. Note that sporocysts glow but the oocyst wall does not (400×). (**G-I** courtesy of Henry S. Bishop, DPDx Team, Centers for Disease Control and Prevention, Atlanta, GA.)

difficulty in maintaining expertise in the interpretation of acid-fast stains. As with *Giardia*, antigen testing is particularly useful for patients with diarrhea who have not traveled outside of the United States. In these patients, antigen testing (usually performed in conjunction with *Giardia* antigen testing) can supplement or replace the traditional subjective O&P examination with acid-fast staining (Cama & Mathison, 2015).

NAATs are also becoming more widely available and have been proven to be more sensitive than antigen testing (Cama & Mathison, 2015). At the time of this writing, three NAAT assays are FDA approved for use in the Unites States (see earlier Amebiasis section).

Cyclospora cayetanensis

Like *Cryptosporidium* spp. and the other coccidia, *C. cayetanensis* causes diarrheal disease in immunocompetent patients and severe, prolonged diarrhea and wasting in immunocompromised individuals (Lindsay & Weiss, 2019; Ortega & Sanchez, 2010). Unlike *Cryptosporidium*, it is a parasite of humans only. Therefore, infection is seen associated with ingestion of food or water that has been contaminated with human feces. The parasite has been recovered from patients in several countries, including the United States, and was initially described as a blue-green alga, a cyanobacterium-like body, or a coccidian-like body, among others (Ortega et al., 1993). Infection causes a flu-like illness with nausea, vomiting, weight loss, and explosive watery diarrhea lasting 1 to 3 weeks. The drug of choice for treatment is trimethoprim/sulfamethoxazole (Drugs for Parasitic Infections, 2013).

Oocysts, passed unsporulated, appear as nonrefractile spheres 8 to 10 μm in diameter that contain a cluster of refractile globules enclosed within a membrane when viewed by light microscopy. A total of 1 to 2 weeks is required for sporulation, after which the mature oocyst contains two sporocysts, each with two sporozoites. In trichrome-stained smears, the oocysts appear as clear, round, and somewhat wrinkled objects. Oocysts autofluoresce bright green to intense blue under ultraviolet epifluorescence; thus, this is a helpful method for locating oocysts in stool specimens (Fig. 65.13G). They stain acid-fast with modified acid-fast (Fig. 65.13B) or auramine-O staining techniques, although unstained oocysts ("ghost" forms) may predominate and could be overlooked by inexperienced microscopists. The hot safranin method provides more uniform staining but requires heating of the stain (e.g., via hot plate or microwave), (Visvesvara et al., 1997). Oocysts of *C. cayetanensis* must be differentiated from those of *Cryptosporidium* spp., which stain in a similar fashion but are smaller (4–6 μm) (Cama & Mathison, 2015).

Cystoisospora belli

Cystoisospora belli (formerly known as *Isospora belli*) undergoes both asexual and sexual development in the cytoplasm of small intestine epithelial cells. Sexual development results in the production of oocysts, which are passed in the stool and mature to the infective stage in the environment. Human infections cause diarrhea and malabsorption but are generally self-limited. In patients with AIDS or other immunosuppressive disorders, disease may persist for months or years and may contribute to death (Pritt, 2018; Lindsay & Weiss, 2019). The drug of choice for treatment is trimethoprim/sulfamethoxazole (Drugs for Parasitic Infections, 2013).

Diagnosis is made by finding the unsporulated or partially sporulated oocysts measuring 12 × 30 μm in fecal specimens, usually in direct wet mounts or concentration preparations (Fig. 65.13D). If the unfixed specimen is left at room temperature for 24 to 48 hours, sporulation can occur. The infectious oocyst contains two sporocysts, each with four sporozoites. These oocysts are similar to those of *Cryptosporidium* and *Cyclospora* in that they test positive by safranin and acid-fast (Fig. 65.13C) and, also like *Cyclospora*, the oocysts autofluoresce (Fig. 65.13H) (Cama & Mathison, 2015).

Sarcocystis spp.

Sarcocystis spp. are two-host coccidians in which the sexual phase develops in the intestinal mucosa of carnivorous animals and the asexual, extraintestinal phase occurs in the muscles and tissues of various intermediate hosts. Humans may serve as definitive or intermediate hosts depending on the species of *Sarcocystis*. Intestinal infection with *Sarcocystis hominis* and *Sarcocystis suihominis* is acquired by the ingestion of raw or incompletely cooked beef or pork, respectively, which contains tissue cysts (sarcocysts). Infection usually is asymptomatic, but occasional patients have transient

diarrhea, abdominal pain, or anorexia. Intestinal infection is self-limited because asexual multiplication occurs in the intermediate host and is not repeated in the definitive host. Treatment is not routinely recommended (Drugs for Parasitic Infections, 2013).

The diagnosis of intestinal infection is established by detection of sporulated 25 × 33 μm oocysts in stool; detection can be enhanced by using IV autofluorescence (Fig. 65.13I). Each mature oocyst contains two sporocysts, each of which contains four sporozoites. The oocyst wall is thin and often is not detectable, or has already ruptured, releasing the two sporocysts. These forms, best seen in wet mounts, appear larger than oocysts of *Cryptosporidium*. Trichrome stains are of little value in detecting these parasites. Humans also may serve as intermediate hosts for several unnamed animal species of *Sarcocystis*, in which cysts are found in skeletal and cardiac muscles (Fayer et al., 2015).

INTESTINAL HELMINTHS

Intestinal helminths discussed here include those nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes) that reside as adults in the gastrointestinal tract or live in other locations (liver, lung, or blood) and produce eggs that exit the human body via the intestinal tract. A fourth group, the acanthocephalans (thorny-headed worms) cause rare to uncommon zoonotic disease in humans and will not be discussed further. Sizes for adult helminths vary from 1 mm to more than 10 m in length; sizes for eggs range from 25 to 150 μm (Fig. 65.14).

An understanding of helminth life cycles and zoogeography is critical in knowing which parasite stages may be present in a presumed infection, what organs or tissues may be involved, and when developmental stages may be expected to appear in clinical specimens. Although diagnosis usually depends on finding and identifying an appropriate developmental stage (egg, larva, or adult), some helminthic infections may be diagnosed chiefly on clinical grounds, on the basis of serologic or molecular evidence, or a combination thereof (Mathison & Pritt, 2018).

Certain species have developmental cycles whereby infectious stages can be transmitted directly from person to person (*Enterobius vermicularis* and *Hymenolepis nana*). In others (*Trichuris*, *Ascaris*, and *Trichostrongylus*), an additional maturation period in the environment is required before the parasite egg or larva (in the latter case) is infectious, thus making these latter parasites more common in settings with lack of adequate sanitary and waste treatment facilities. Ingestion of infective stages may also occur incidentally after the ingestion of insects or mollusks (*Dipylidium*, *Hymenolepis*, *Angiostrongylus*), plants (*Fasciolopsis*, *Fasciola*), or animal tissues (*Trichinella*, *Taenia*, *Dibothriocephalus*, *Clonorchis*, *Opisthorchis*, *Paragonimus*, *Heterophyes*, *Metagonimus*, and *Nanophyetus*). In some cases, larval parasite stages may directly penetrate the skin (hookworms, *Strongyloides*, *Schistosoma*).

Recovery and identification of helminth eggs and larvae in stool, urine, or sputum requires a systematic approach and appropriate training of the individuals performing the evaluations. The size of the eggs and larvae is an especially important characteristic; measurement often requires a properly calibrated ocular micrometer. External characteristics of eggs that should be documented include their shape, their wall thickness, and the presence or absence of a mamillated covering, operculum, opercular shoulders, abopercular knob, polar plugs, or spines. Egg development (embryonated, unembryonated) and the presence or absence of hooklets, which are characteristic of cestodes, should also be noted. The examiner also needs to have an appreciation for the large variety of artifacts detected in human feces that may mimic parasite eggs and larvae (Ash & Orihel, 2007; Pritt, 2014).

NEMATODES

Nematodes (roundworms) are common worms that inhabit the human intestinal tract, blood, and tissues. Some attach to intestinal mucosa by means of specialized mouthparts at the anterior end (e.g., hookworms, whipworm). Others move freely in the lumen of the bowel (e.g., pinworm, *Ascaris lumbricoides*); thus, they may be more likely to end up in ectopic locations. Nematodes have elongated, unsegmented bodies covered with an acellular cuticle and possess a full digestive tract. Most are dioecious (whereby male and female worms are individual animals), unlike the cestodes and some of the trematodes, which are hermaphrodites. Identification of characteristic eggs in stool is used for diagnosis of all intestinal nematode infections with the exception of *Strongyloides stercoralis*, in which only first-stage (rhabditiform) larvae are typically seen in stool, and pinworm, in which eggs are deposited onto the perianal skin folds and are not commonly found in stool. Of the intestinal nematodes, only the eggs of pinworm become infectious within 4 to 6 hours of being laid. The eggs of the other intestinal nematodes require an incubation period of several weeks in the soil to become infectious. Soil-transmitted helminth infections are identified as a neglected tropical disease and are seen in areas of

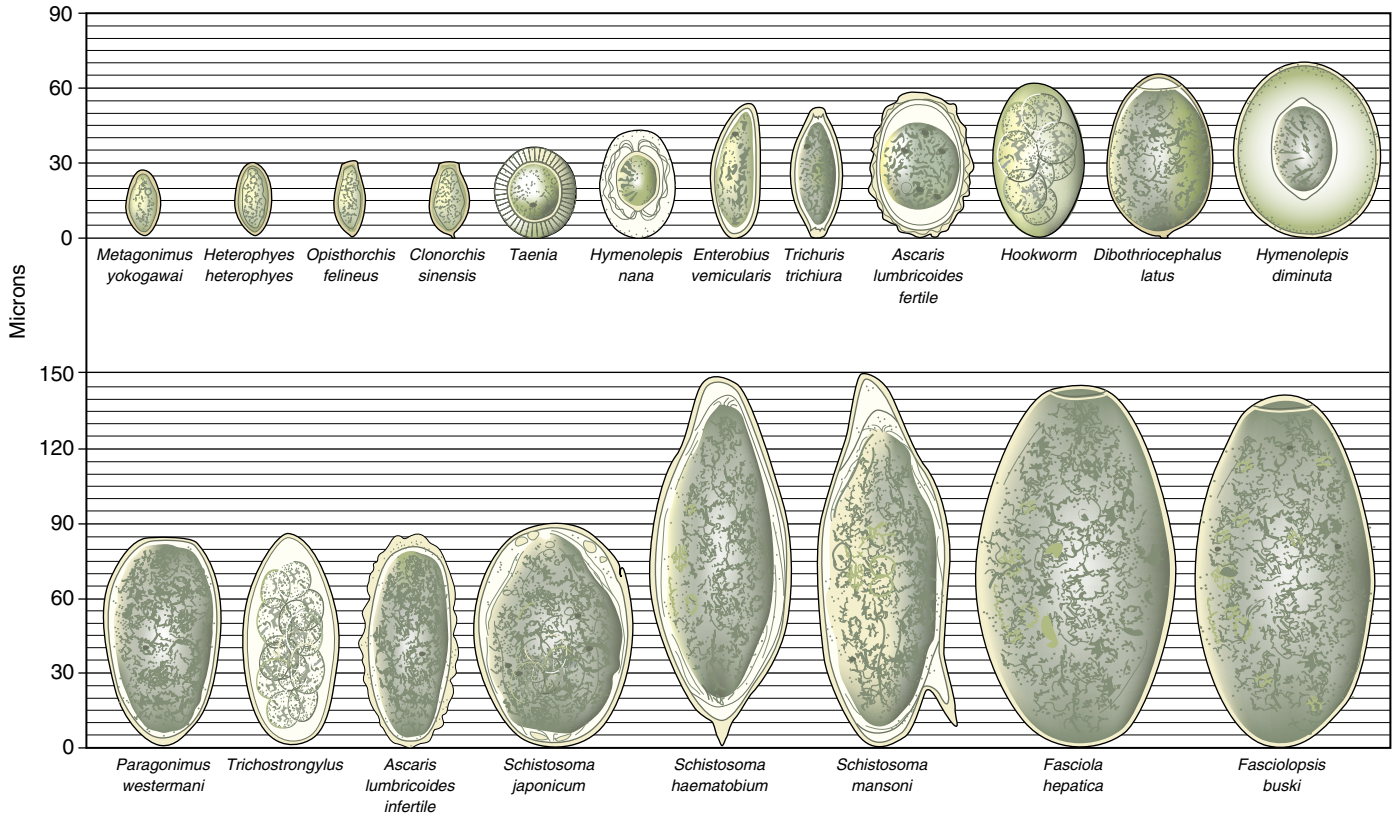


Figure 65.14 Relative sizes of helminth eggs. (Courtesy of Centers for Disease Control and Prevention, Parasitology Training Branch, Atlanta, GA.)

the world where sanitation is poor. Up to one-quarter of the world's population is estimated to be infected with soil-transmitted helminths (WHO, 2019). This section covers intestinal nematodes. Nematodes that are usually detected in intestinal biopsy specimens or extraintestinal sites are covered in the Tissue Helminths section later in the chapter.

Enterobius vermicularis (Pinworm Infection)

Enterobiasis is the most common helminthic infection in children of all social strata in the United States, Canada, and Europe. Although it is primarily a parasite of young children, rapid maturation of the egg allows it to be readily transmitted from person to person in both family and institutional settings. Male and female worms reside primarily in the cecum and adjacent areas. Females measure up to 13 mm in length and have a pointed posterior end that gives rise to their common name, the pinworm. Both sexes have lateral alae that are best seen in the cervical region and in cross-section as well as a prominent esophageal bulb (Figs. 65.15A and 65.15C).

Although males are rarely seen in clinical specimens, females may be found on the surface of a stool specimen or on the perianal skin, especially at night, where eggs are deposited. Eggs are colorless and ovoid with one side flattened and measure 20 to 40 μm wide by 50 to 60 μm long (see Fig. 65.15B). They are infective within hours and when ingested complete development to the gravid adult stage within 1 month (the prepatent period).

Although infection may be asymptomatic, children often suffer from nocturnal pruritus ani, irritability, and loss of sleep. Enterobiasis should be ruled out early in the evaluation of enuresis. Treatment is with albendazole, mebendazole, or pyrantel pamoate (Drugs for Parasitic Infections, 2013). Adult worms may also migrate to unusual sites, such as the vagina, fallopian tubes, or peritoneal cavity. Their egg-laying behavior and ultimate death in

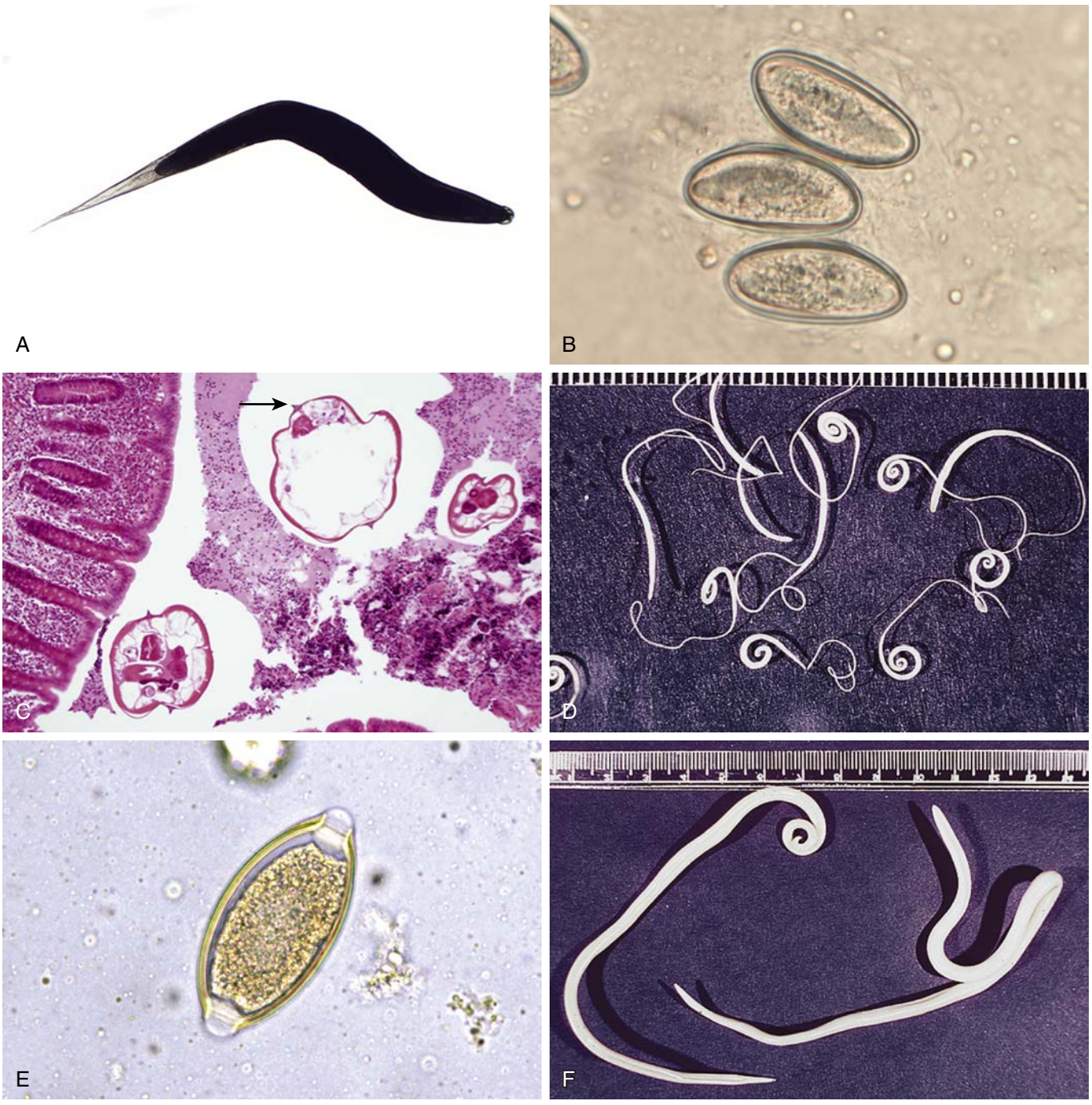


Figure 65.15 All direct examination with brightfield illumination except as noted. **A,** Adult female pinworm, *Enterobius vermicularis*, with pointed "pin-like" posterior end. Prominent anterior inflations (alae) are also seen (4×). **B,** Numerous eggs of *E. vermicularis* as seen on a specialized "pinworm paddle" (400×). **C,** Cross-sections of adult *E. vermicularis* in the appendix; note characteristic lateral alae *(arrow)* (H&E; 100×). **D,** Adult whipworms, *Trichuris trichiura*; note females with straight tails and males with coiled tails. **E,** Egg of *T. trichiura* (400×). **F,** Adult female and male *Ascaris lumbricoides*, the largest human intestinal roundworm. The female has a straight tail, whereas the male has a coiled tail.

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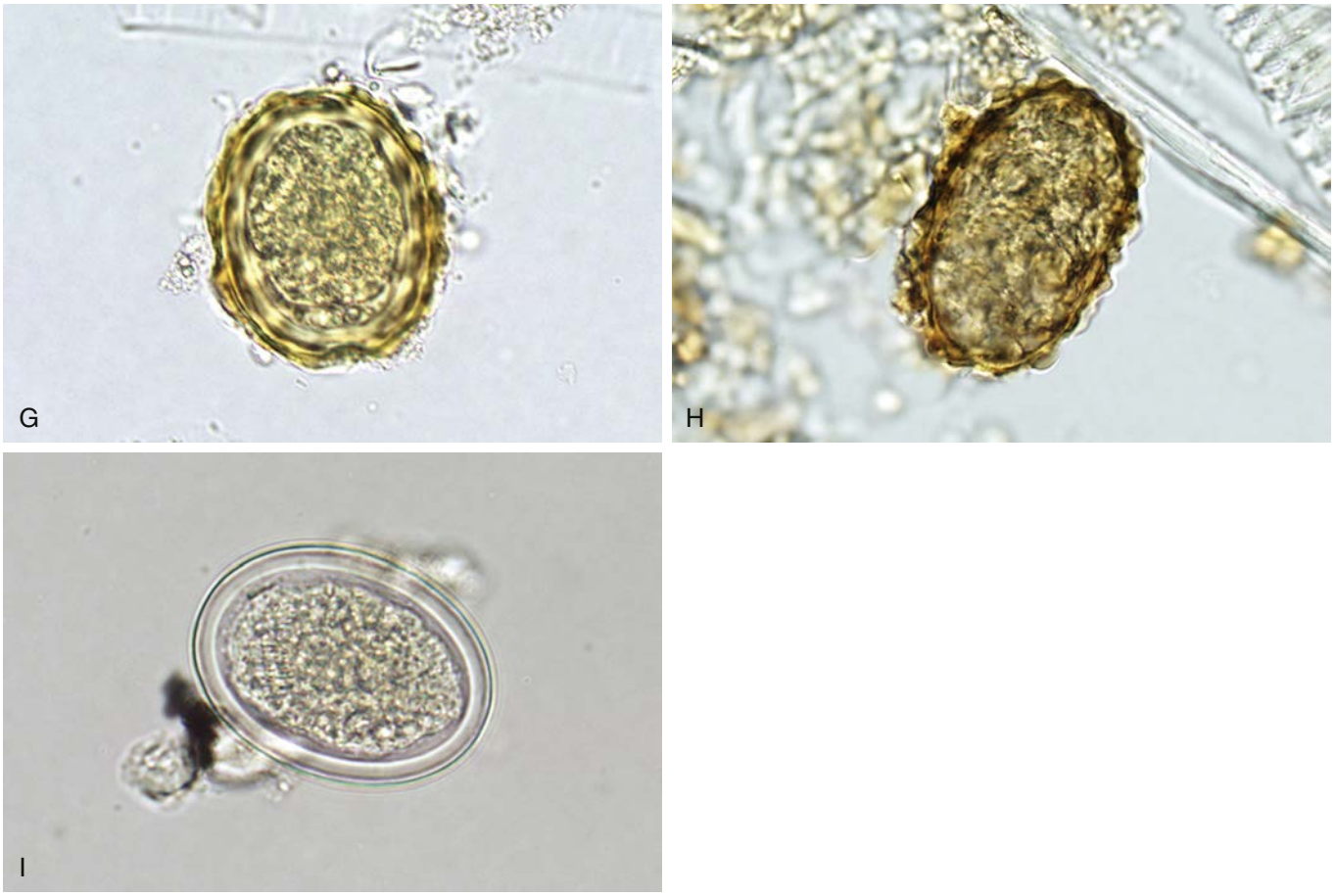

Figure 65.15, cont'd **G,** Fertile, unembryonated egg of *A. lumbricoides* (400×). **H,** Infertile egg of *A. lumbricoides* (400×). **I,** Decorticated fertile egg of *A. lumbricoide* (**D** and **F** from Zaiman H, editor: A pictorial presentation of parasites: a cooperative collection; https://www.astmh.org/education-resources/zaiman-slide-library.)

these locations may provoke inflammatory, granulomatous reactions (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018).

Recovery of eggs or, less commonly, adults from the perianal skin is usually done using the cellulose tape technique or commercially available collection kits (see Fig. 65.2) first thing in the morning before the patient bathes or defecates (Ash & Orihel, 2007; Garcia, 2016). Only 5% to 10% of cases are detected using routine stool examination since the eggs are laid on the skin rather than being secreted into the colonic lumen (Garcia, 2016).

Trichuris trichiura (Whipworm Infection)

Trichuriasis is common worldwide in tropical and subtropical regions with inadequate sanitation and waste treatment facilities. Adult worms are found in the large intestine, especially the cecum, but in heavy infections they can be found throughout the colon and rectum. Males and females measure up to 50 mm in length and remain attached to the intestinal mucosa by the long, slender anterior end, while the thicker posterior end hangs free in the lumen. Female worms are elongate, whereas the tails on males are coiled (Fig. 65.15D). *Trichuris* has a direct life cycle in which eggs are passed in stool unembryonated and require several weeks under appropriate soil conditions to mature to the infective stage. When fully embryonated eggs are ingested, larvae are released and mature into adults in the colon, where they attach and survive up to 10 years.

Light infection usually is asymptomatic, but when larger numbers (>300 worms) are present, diarrhea or symptoms of dysentery may develop in association with dehydration and anemia. Rectal prolapse may occur in heavily infected children (Bethony et al., 2006). Treatment is with albendazole, mebendazole, or ivermectin (Drugs for Parasitic Infections, 2013).

Diagnosis is made by finding typical eggs in direct fecal smears or with concentration techniques. The eggs are barrel shaped with refractile plugs at both ends and usually measure 50 to 55 μm long by 22 to 24 μm wide (Fig. 65.15E). Egg quantitation techniques occasionally may be requested, primarily in endemic settings, to assess infection intensity, therapeutic efficacy, and reacquisition rates of parasites.

Capillaria philippinensis

This parasite, normally found in fish-eating birds, infects humans who ingest raw or incompletely cooked fish that contain infective larvae in their flesh. Although first described in persons from the Philippines, and later Thailand, occasional cases have been reported in Asia, the Middle East, and South America (Cross, 1992; Ash & Orihel, 2007). The parasites can cause autoinfection, resulting in chronic diarrhea. Treatment is with mebendazole or albendazole (Drugs for Parasitic Infections, 2013). Infected individuals may pass eggs, larvae, and even adult worms in their feces. Eggs resemble those of *Trichuris*, although they measure 36 to 45 μm in length by 21 μm in width and have thick, radially striated shells and bipolar protuberances that are inconspicuous (Fig. 65.16F) (Ash & Orihel, 2007).

Ascaris lumbricoides (Ascariasis)

This is the largest nematode that infects the intestinal tract of humans and is one of the most common of the intestinal roundworms, infecting an estimated 1.3 billion individuals worldwide (John & Petri, 2006). Infection occurs primarily in areas with inadequate sanitation and waste treatment facilities. As with *Trichuris*, it is especially common in children, who are also more likely to harbor heavy infection. In the United States, infection is often associated with pigs, which serve as a reservoir host.

Adult *Ascaris* live primarily in the duodenum and proximal jejunum. Females measure up to 35 cm in length by 6 mm in diameter. The male is somewhat smaller and has a ventrally curved tail, unlike the female (Fig. 65.15F). Both adult and immature worms can be identified by the presence of three prominent lips at the anterior end.

Females produce approximately 200,000 eggs per day, which are unembryonated when passed and require 4 to 6 weeks in a satisfactory environment to become infective. Following ingestion, eggs hatch in the intestine, and larvae penetrate the mucosa to gain access to the bloodstream. They are carried to the lungs and mature briefly in the alveolar capillary bed before entering the alveoli. Respiratory clearance mechanisms move the larvae to the epiglottis, where they are swallowed and grow to adulthood in the small bowel. Development from embryonated egg to adult takes approximately 2 months.

Symptoms of ascariasis vary from asymptomatic infection to severe disease. Migration of large numbers of larvae through the lungs during the initial phase of infection can cause *Ascaris* pneumonitis or Loeffler syndrome, characterized by bilateral diffuse, mottled pulmonary infiltrates and mild

bronchitis associated with peripheral eosinophilia. The syndrome is rare and usually occurs in individuals who have been previously exposed to *Ascaris* (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018). Treatment is with albendazole, mebendazole, or ivermectin (Drugs for Parasitic Infections, 2013).

Light infections are usually asymptomatic, whereas heavy infection may produce varying degrees of abdominal pain and diarrhea. Intestinal obstruction may also occur with a mass of worms, especially in children. Even a small number of worms are cause for concern because of their ability to invade ectopic sites such as the common bile duct and liver, appendix, and stomach. Fever or drug therapy may stimulate migration. In endemic areas, anthelmintics often are prescribed before anesthetics are used in elective surgery.

Infection is diagnosed by demonstrating eggs in feces or on recovery of an adult that has been passed or vomited. The large number of eggs produced each day makes detection of even a single worm probable. A count of fewer than 20 eggs per slide (2 mg of feces) indicates light infection, and a count of more than 100 eggs per slide indicates heavy infection.

Fertile *Ascaris* eggs are round to slightly oval with a yellow-brown, irregular external mamillated layer and a thick shell. Eggs that have lost their mamillated layer are described as *decorticated* and may superficially resemble hookworm eggs. Fertile eggs are passed unembryonated and measure approximately 55 to 75 μm long by 35 to 50 μm wide (Figs. 65.15G and 65.15I). Single females may produce unfertilized eggs, which are larger and more elongate (up to 90 μm in length) and have a thinner shell with irregular mammillations (Fig. 65.15H). These eggs lack organized internal contents and are filled with irregularly sized globules.

Trichostrongylus spp.

Human disease caused by *Trichostrongylus* spp. represents a zoonotic infection because these parasites principally infect large herbivores, such as sheep, cattle, and goats. The genus occurs worldwide, and several species may infect humans, including *Trichostrongylus colubriformis*, *Trichostrongylus orientalis*, *Trichostrongylus axei*, and *Trichostrongylus brevis*. Adult worms inhabit the small bowel and produce eggs that mature outside of the body. Larvae emerge and crawl about on soil and vegetation, where they are available to be ingested by potential hosts. Unlike hookworms, they do not invade skin directly, nor does the life cycle involve a migratory phase through the lungs. Infection usually is light and asymptomatic, but heavy infection may produce abdominal pain and diarrhea, usually with eosinophilia. Treatment is with pyrantel pamoate, mebendazole, or albendazole (Drugs for Parasitic Infections, 2013). Eggs resemble those of hookworms but are longer and narrower, measuring 78 to 98 μm by 40 to 50 μm, and are slightly tapered at one end (Fig. 65.16B).

Necator americanus, Ancylostoma duodenale, and *A. ceylanicum* (Hookworm Infection)

Hookworms, which are among the more common helminths known to infect humans, occur in tropical and subtropical regions and some temperate areas where there are inadequate sanitation and waste treatment facilities. *Necator americanus* is found in the United States and in other areas of the world and frequently overlaps in distribution with *Ancylostoma duodenale*. In Southeast Asia, *A. ceylanicum* is a common cause of intestinal hookworm infection (Ngui et al., 2014). Given their overlapping distributions, it is inaccurate to classify *A. duodenale* as an "Old World" hookworm and *N. americanus* as a "New World" hookworm; use of these terms should be avoided.

Adult females measure up to 12 mm in length and the males slightly less. Males are readily distinguished by the fan-shaped copulatory bursa at the posterior end. The anterior end of hookworms is modified into a buccal capsule that contains teeth or cutting plates. Both sexes attach to the mucosa of the small intestine, where they may reside for up to 18 years (Ash & Orihel, 2007).

Eggs are passed in feces and develop rapidly, depending on prevailing conditions. Rhabditiform larvae are released and develop into the infective filariform stage in about 7 days. On contact with an appropriate host, the larvae penetrate the skin, gain access to the host's circulation, travel to the lungs, and move up the tracheobronchial tree to be swallowed. On maturation in the small intestine, oviposition begins. Although the life cycles of both species are similar, *Ancylostoma* can mature directly to the adult stage in the intestine if infective larvae are ingested.

Hookworms can produce clinical symptoms in the skin at the site of larval penetration. This condition, known as *ground itch*, is characterized by inflammation, redness, and blister formation, along with intense itching. Migration of large numbers of larvae through the lungs may produce Loeffler syndrome, as described earlier for *A. lumbricoides*. Depending on the worm burden, intestinal infection can result in gastroenteritis with abdominal pain, diarrhea, and nausea. Hookworms are known for their ability to produce chronic blood loss with secondary iron-deficiency anemia. The presence of each adult *A. duodenale* can result in the loss of 0.15 to 0.25 mL of blood per day compared with 0.03 mL for each *N. americanus*. Development of children can be severely affected by chronic infection. Blood loss and the number of hookworms present correlate with the number of eggs per gram of stool, which may help the clinician determine when to initiate therapy in individuals living in endemic areas (Farrar et al., 2013). Treatment is with albendazole, mebendazole, or pyrantel pamoate (Drugs for Parasitic Infections, 2013).

Diagnosis is made by finding the characteristic thin-shelled eggs in feces. These eggs are partially embryonated when passed and measure 58 to 76 μm in length by 36 to 40 μm wide (Fig. 65.16A). Embryonated eggs or free rhabditiform larvae may be found in unpreserved specimens that are not examined promptly, and the larvae must be differentiated from the similar-appearing larvae of *Strongyloides stercoralis*. This can be accomplished using morphologic features; hookworm rhabditiform larvae have a longer buccal canal than those of *S. stercoralis* and inconspicuous genital primordium (Fig. 65.17). Hookworm eggs may need to be differentiated from those of *Trichostrongylus* spp. (which are longer and more pointed) and those of plant parasitic nematodes, especially *Heterodera* spp. (which are longer, have blunt ends, and are often asymmetric).

Although adult hookworms can be differentiated on the basis of their mouthparts and the copulatory bursa in males, eggs of human hookworms are indistinguishable. In direct wet mounts, egg counts of fewer than five eggs per coverslip denote light infection that is unlikely to result in anemia, whereas more than 25 eggs per coverslip denotes heavy infection that is likely to be associated with symptoms.

Strongyloides stercoralis (Strongyloidiasis)

Strongyloidiasis occurs in many areas in the tropics and subtropics, but this infection also is reported in temperate zones and has historically been endemic in areas of southeastern United States (e.g., regions of Appalachia with lack of adequate sanitary facilities and indoor plumbing). Adult females are 2 to 3 mm long and live in the mucosa of the duodenum, where they reproduce parthenogenetically (i.e., without a male; Fig. 65.16E). Males do not occur in the parasitic phase of the life cycle. The eggs hatch primarily in the small bowel, releasing first-stage (L1) or rhabditiform larvae, which then are passed in the feces (eggs are rarely found in stool O&P examinations). In this direct cycle, the rhabditiform larvae metamorphose into infective third-stage (L3) filariform larvae in the soil. These infective larvae readily penetrate the skin of exposed individuals; they migrate randomly throughout the body, eventually settling in the small intestine, where they mature into adults. Under appropriate soil conditions of high humidity, an indirect cycle may appear transiently, in which the newly deposited larvae develop into a free-living generation consisting of reproductive males and females. Eggs produced by this generation develop into filariform larvae that again are infective for humans. A third variation in the life cycle of *Strongyloides* involves autoinfection, in which maturation to the filariform stage is completed within the intestinal tract, with subsequent reinvasion of bowel mucosa or perianal skin.

Disease presentation is variable and may depend on the strain acquired. Early migration of filariform larvae may produce irritation, redness, and pruritus at the site of entry. Later migration through the lungs may produce Loeffler syndrome as previously discussed, with *A. lumbricoides* and the hookworm (Purtilo et al., 1974). The presence of intestinal symptoms is related to the intensity of the infection. The affected individual may have symptoms of peptic ulcer, abdominal pain, and diarrhea. A malabsorption syndrome has been reported with chronic infection.

The ability of the parasite to autoinfect may result in persistence of the infection for decades, as was recognized in allied troops who were held as prisoners of war in Southeast Asia during World War II and found to have active infection decades later (Gill & Bell, 1979). In otherwise healthy patients, autoinfection is generally asymptomatic but may produce larva currens (linear urticarial lesions). In immunocompromised individuals (e.g., patients with human T lymphotropic virus type 1 [HTLV-1] infection, transplant recipients, those receiving chemotherapy, end-stage alcoholics, and severely malnourished individuals), autoinfection may result in a life-threatening hyperinfection syndrome caused by rapid multiplication of the parasite (Meyers et al., 2000). Severe pneumonia is often a presenting manifestation of hyperinfection, followed by marked diarrhea, enteritis, septicemia, and recurrent gram-negative meningitis. Bacteria and bacterial meningitis occur secondary to carriage of intestinal bacteria by migrating larvae into the bloodstream and tissues. Patients who have lived in endemic areas should be screened for *S. stercoralis* prior to receiving immunosuppressive therapy (CDC, 2018c). Unlike infection with the other intestinal nematodes, the drug of choice for strongyloidiasis is ivermectin (Drugs for Parasitic Infections, 2013).

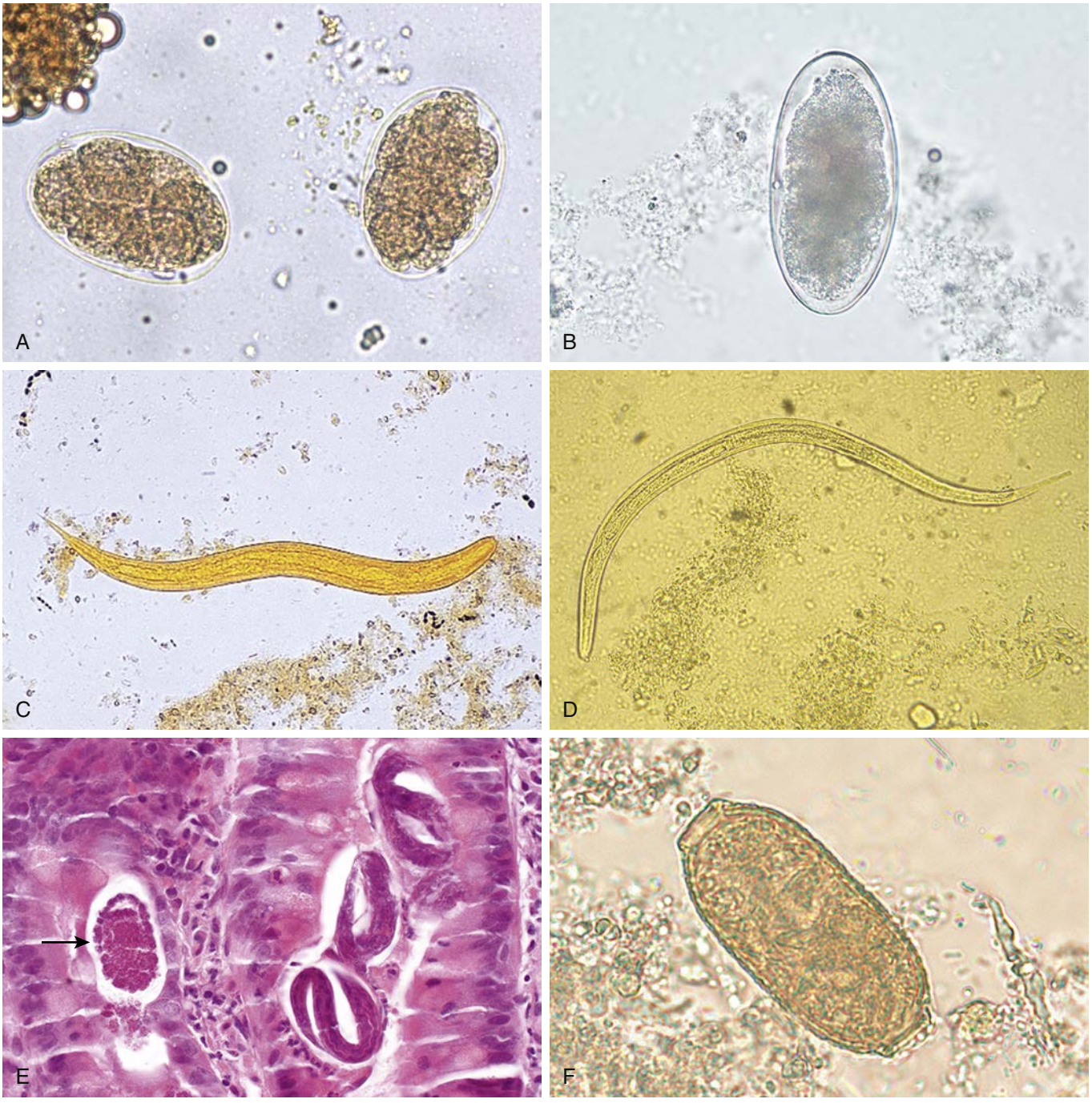

Figure 65.16 **A,** Hookworm (*Ancylostoma* sp. or *Necator americanus*) eggs (400×). **B,** Egg of *Trichostrongylus* sp. (400×). **C,** *Strongyloides stercoralis* rhabditiform (L1) larva in stool (iodine stain; 200×). **D,** Filariform (L3) larva of *S. stercoralis* in a bronchial wash (400×). **E,** Massive *S. stercoralis* infection in the duodenal mucosa. Note the presence of three larvae *(right)* and an egg *(arrow)* (H&E; 200×). **F,** *Capillaria philippinensis* egg in stool (400×).

Continued

Diagnosis is made on recovery and identification of typical rhabditiform larvae in stool specimens, although the routine O&P examination does not always reveal their presence and up to seven stool specimens may need to be examined for optimal sensitivity with this method (Garcia, 2016). When seen, *S. stercoralis* rhabditiform larvae must be differentiated from those of hookworms by their short buccal cavity and a prominent genital primordium (see Fig. 65.16C and 65.17). *S. stercoralis* filariform larvae may also be seen in respiratory and other extraintestinal specimens in disseminated disease. They have a notched tail and an esophagus approximately half the length of the body (Fig. 65.16D). Either stage of larvae is readily seen in fresh saline wet mounts under low power (e.g., 10×). If infective filariform larvae are detected in a recently passed stool specimen or in any extraintestinal specimens, superinfection should be suspected and reported immediately to the clinical team (Sheorey et al., 2019).

Examination of duodenal aspirates or string test specimens may be helpful in suspicious cases in which routine stool examinations are nonproductive, although they are not commonly used. The agar culture method (see Fig. 65.3) or one of the coproculture techniques (see the Laboratory Methods section earlier in the chapter) may also demonstrate the infection and provide highest sensitivity for detection of *S. stercoralis* from fecal specimens (Ash & Orihel, 2007; Garcia, 2016). Serologic tests are useful when infection is suspected but cannot be demonstrated by other methods; they are also useful for detecting evidence of prior infection. EIA and other tests display good sensitivity and specificity, although cross-reactions may appear with filariasis and some other nematode infections. These tests generally do not differentiate between past and current infection but may be useful in monitoring therapy (Wilkins & Nutman, 2015).

CESTODES

Cestodes, commonly referred to as tapeworms, are ribbon-like flatworms that live in the intestinal tract of vertebrate definitive hosts as adults and in the tissues or body cavities of various intermediate hosts as larvae. Depending on the species, humans can serve as definitive or intermediate

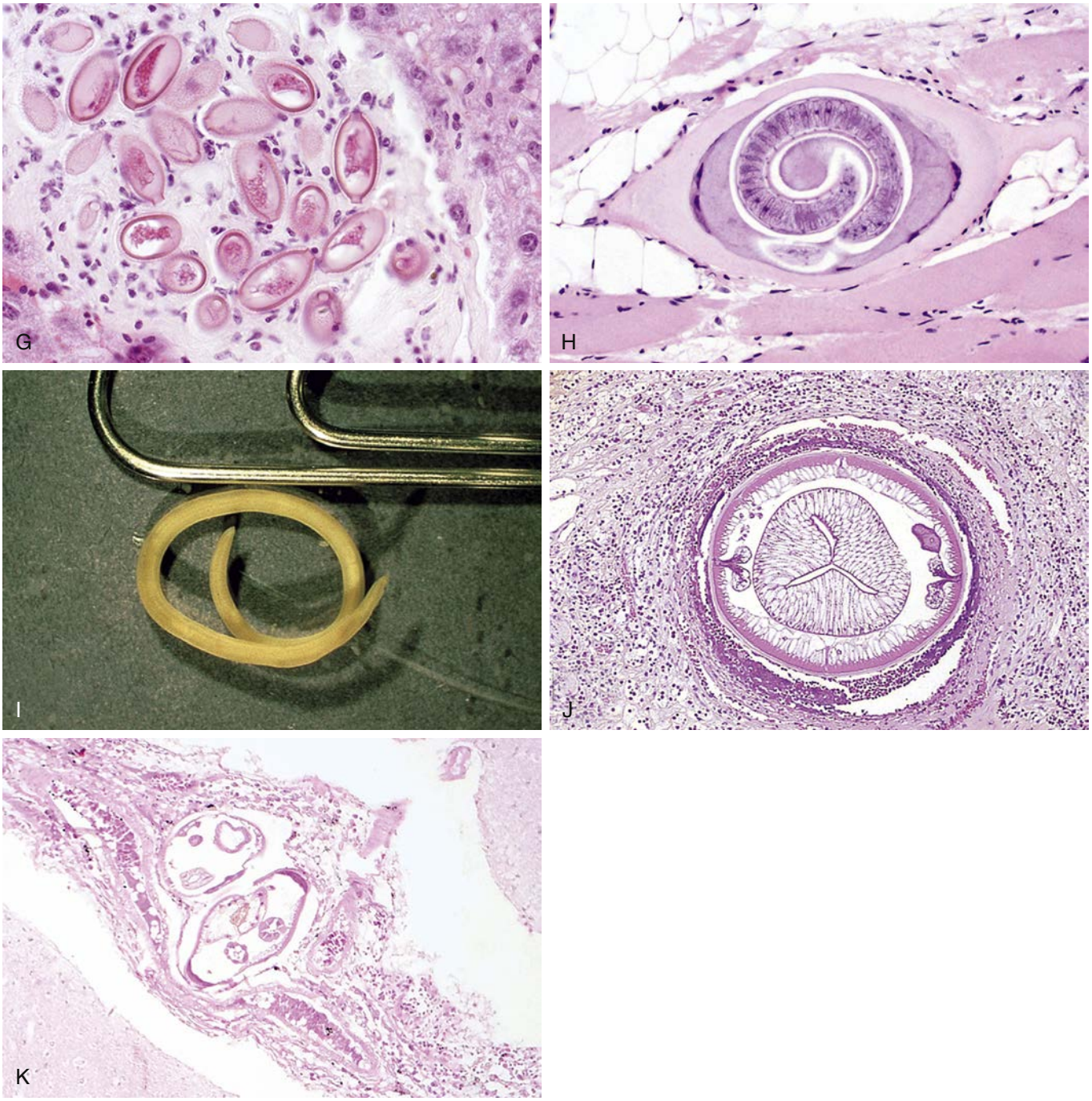

Figure 65.16 **G,** Eggs of *Capillaria hepatica* in a liver biopsy (H&E; 100×). **H,** *Trichinella* sp. cross-section of a larva in gastrocnemius muscle (H&E; 100×). **I,** Regurgitated anisakid nematode following consumption of fish. **J,** *Anisakis* sp., cross-section of larva found in small bowel following surgery for acute obstruction (H&E; 400×). **K,** Cross-sections of L4 larvae of *Angiostrongylus cantonensis* in brain tissue (H&E; 100×).

hosts and, in the case of *Taenia solium*, as both. Adult tapeworms attach to the intestinal mucosa by means of a scolex (head) that may display suckers, grooves (bothria), or a rostellum (knob-like protrusion) with hooks, depending on the species. The body of the worm (strobila) comprises an actively growing neck region that produces immature proglottids. These proglottids then undergo sequential development into mature and, eventually, gravid stages as they are pushed toward the posterior end with the formation of new immature proglottids. Each proglottid has a complete set of male and female gonads and is capable of producing fertile eggs, although sexual reproduction between two individual worms is the norm. Eggs of most cestodes infecting humans (*Dibothriocephalus* and related being the exception) may be readily differentiated from those of other helminths by the presence in each of a six-hooked embryo (oncosphere). Depending on the species, eggs are released directly into the fecal stream or are passed in intact proglottids. It is not uncommon in some species for long lengths of strobila to be passed intact or for proglottids to actively migrate out of the anus. Large species of *Taenia* and *Dibothriocephalus* may grow to 25 feet or longer and may live for 20 years. The treatment of choice for adult tapeworm infection is praziquantel, with niclosamide as an alternative drug (Drugs for Parasitic Infections, 2013).

Cestode larval stages develop to the infective stage in the tissues of invertebrate or vertebrate intermediate hosts, depending on the species, and complete their life cycle when ingested by a definitive host. Larval stages of several species may infect humans, causing cysticercosis, hydatidosis, sparganosis, and coenurosis. These conditions are covered more fully in the Tissue Helminths section later in the chapter. Unlike treatment for intestinal cestodes, treatment of larval tissue cestodes is with albendazole (Drugs for Parasitic Infections, 2013).

Taenia saginata (Taeniasis)

Humans are the sole definitive host for *Taenia saginata*, the beef tapeworm. Although it is distributed worldwide, infection is most common in the Middle East, Africa, Europe, Asia, and Latin America. It occurs rarely and sporadically in the United States, seen primarily in immigrants.

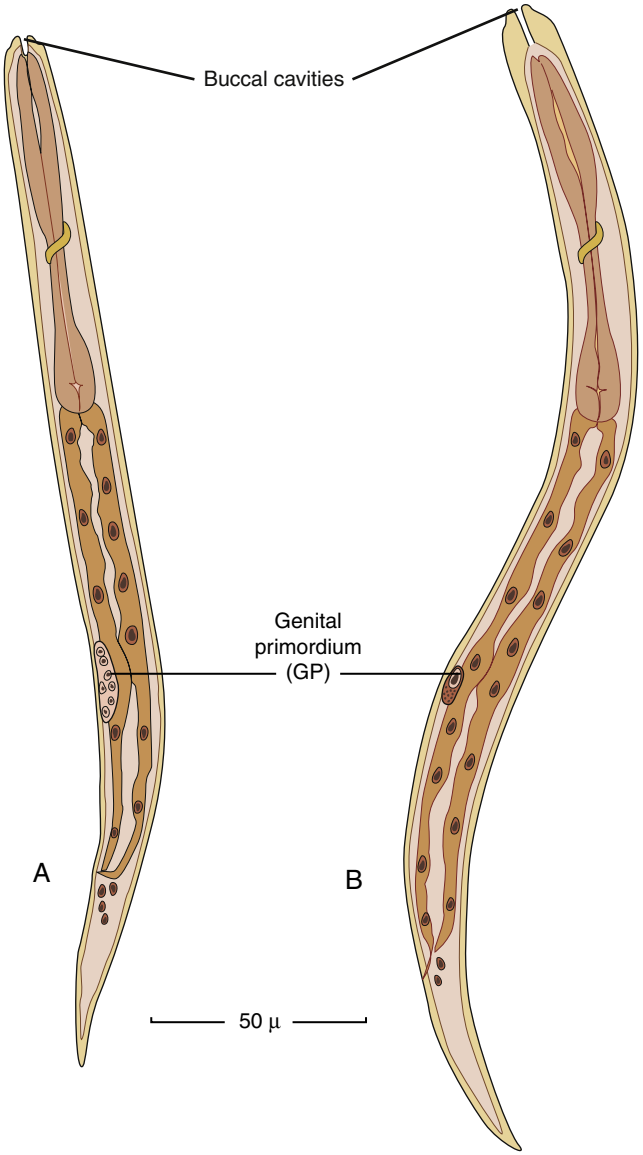

Figure 65.17 Hookworm and *Strongyloides stercoralis* larvae. **A,** *S. stercoralis* rhabditoid larva in human stools. Note the short size of the buccal cavity and the large genital primordium (GP). **B,** Hookworm rhabditoid larva as seen if there is a delay in processing stool stored at room temperature. The buccal cavity is longer and the GP is obsolete.

Larval cysticerci develop in the tissues of cattle that graze on land contaminated by human waste. When humans ingest infected raw or incompletely cooked beef, the cysticercus develops into a reproductive adult in the small intestine in 2 to 3 months. It uses a specialized scolex to attach to the small intestinal wall and then elongates as proglottids are produced, as described earlier. Most infected individuals are asymptomatic; when present, they commonly include abdominal discomfort and diarrhea. Unlike *T. solium* (discussed later), the eggs of *T. saginata* are not infectious to humans, and their ingestion does not result in human cysticercosis. Treatment is with praziquantel (Drugs for Parasitic Infections, 2013).

Diagnosis is made by finding characteristic eggs in the stool or shed proglottids. Eggs are spherical and measure 31 to 43 µm in diameter (Fig. 65.18A). The shell is thick, radially striated, and contains a six-hooked embryo. Eggs of all *Taenia* spp. are indistinguishable and should therefore be reported only as *Taenia* spp. eggs.

Species identification may only be made by microscopic examination of the proglottids or, more rarely, the scolex (following administration of an anthelminthic agent), or by NAATs. Proglottids of taeniids have a characteristic single lateral protrusion known as the *genital pore*. Careful injection of India ink through the genital pore using a tuberculin needle and syringe, following clearing in lactophenol, may succeed in outlining the uterus. In many circumstances, pressing an unpreserved proglottid between two slides, with or without prior clearing, will also adequately highlight the uterus and allow for species identification. The gravid uterus of *T. saginata* has 15 to 20 primary lateral branches (counted on one side only), whereas that of *T. solium* has 7 to 13 primary lateral branches (Figs. 65.18F and 65.19). Proglottids may also be cleared overnight in glycerol or stained with carmine or hematoxylin using published procedures (Ash & Orihel, 2007). If recovered, the scolex of *T. saginata* can be identified by the presence of four suckers and the absence of hooks on the crown or rostellum. When handling *Taenia* proglottids, it is important to remember that the eggs contained within may cause cysticercosis if ingested (if *T. solium*); thus, utmost care is warranted.

Taenia asiaticus (formerly considered a subspecies of *T. saginata*) cycles between humans and pigs in Southeast Asia. The proglottids of *T. asiaticus* are similar to those of *T. saginata* but the scolex has an armed rostellum, similar to *T. solium*. This species is not known to cause cysticercosis in humans (Ale et al., 2014).

Taenia solium (Taeniasis)

Taenia solium, the pork tapeworm, is most common in Europe, especially in Eastern Europe, Latin America, China, Pakistan, and India. It is encountered in the United States on occasion, most often in immigrants. Infection with the adult tapeworm (taeniasis) is acquired by eating raw or incompletely cooked pork containing cysticerci). Most patients with taeniasis due to *T. solium* are asymptomatic. Symptoms, if present, are identical to those of *T. saginata* infection. Treatment is with praziquantel (Drugs for Parasitic Infections, 2013). Importantly, accidental ingestion of *T. solium* eggs from one's own adult tapeworm or from contaminated food may result in cysticercosis. Past outbreaks of cysticercosis in the United States have been associated with food preparers with taeniasis due to *T. solium* (Schantz et al., 1992; CDC, 2018b). Additional details on cysticercosis may be found in the Tissue Helminths section later in the chapter.

Procedures used for diagnosis of intestinal *T. solium* infection are identical to those used for *T. saginata* infection, although certain morphologic differences are apparent. The scolex of *T. solium* has four suckers and, unlike *T. saginata*, a rostellum armed with two rows of hooks. Gravid proglottids have 7 to 13 primary lateral uterine branches on each side of the uterine stem (see Fig. 65.19).

Hymenolepis nana (Hymenolepiasis)

Hymenolepis nana, known as the dwarf tapeworm, has a worldwide distribution and is regarded as the most frequently recovered cestode species seen in the United States. It is a common parasite in mice and is the smallest cestode to infect humans, measuring up to 4.0 cm in length. The scolex has an armed rostellum containing a single row of hooks, and the proglottids have all of their genital pores located on the same side of the strobila (see Fig. 65.19). The life cycle may be direct, through the ingestion of infectious eggs, or indirect, through the ingestion of intermediate hosts (usually grain beetles) containing cysticercoid larvae. In the former instance, eggs may be passed directly from person to person, usually among children (highest likelihood), or may be ingested in food, such as grain products that are contaminated by grain beetles or possibly rodent droppings (Ash & Orihel, 2007).

Ingested eggs hatch in the intestine and embryos penetrate the mucosa, where they mature into cysticercoid larvae. They subsequently emerge and reattach to the intestinal wall to complete their development into adult tapeworms in 2 to 3 weeks. Therefore, humans serve as both the intermediate and definitive host for this parasite. Internal autoinfection may occur in some individuals in whom eggs hatch shortly after being discharged from the worm and rapidly invade the intestinal wall without leaving the body. Such a mechanism is thought to be responsible for the occasional case of massive infection.

Symptomatic infection, characterized by abdominal pain, diarrhea, anorexia, and irritability, may develop in patients with large numbers of worms. Treatment is with praziquantel (Drugs for Parasitic Infections, 2013). Diagnosis is made by recovery from stool of the oval, thin-shelled, colorless eggs, which measure 30 to 47 µm in diameter (Fig. 65.18B). They contain a centrally located, six-hooked embryo (oncosphere), which is separated from the outer shell by a clear space. This embryo displays two polar thickenings from which thin filaments arise and extend into the clear space between the embryo and outer shell. Rarely, intact strobila may be recovered if the stool is closely examined or by colonoscopy.

Hymenolepis diminuta (Hymenolepiasis)

The rat tapeworm, *Hymenolepis diminuta*, is cosmopolitan in distribution and rarely infects humans. Infection is infrequent because of the obligate need for an arthropod intermediate host, in which the cysticercoid larvae develop. Human infection usually occurs following the accidental ingestion of infected beetles that contaminate grain or cereal products. Adult

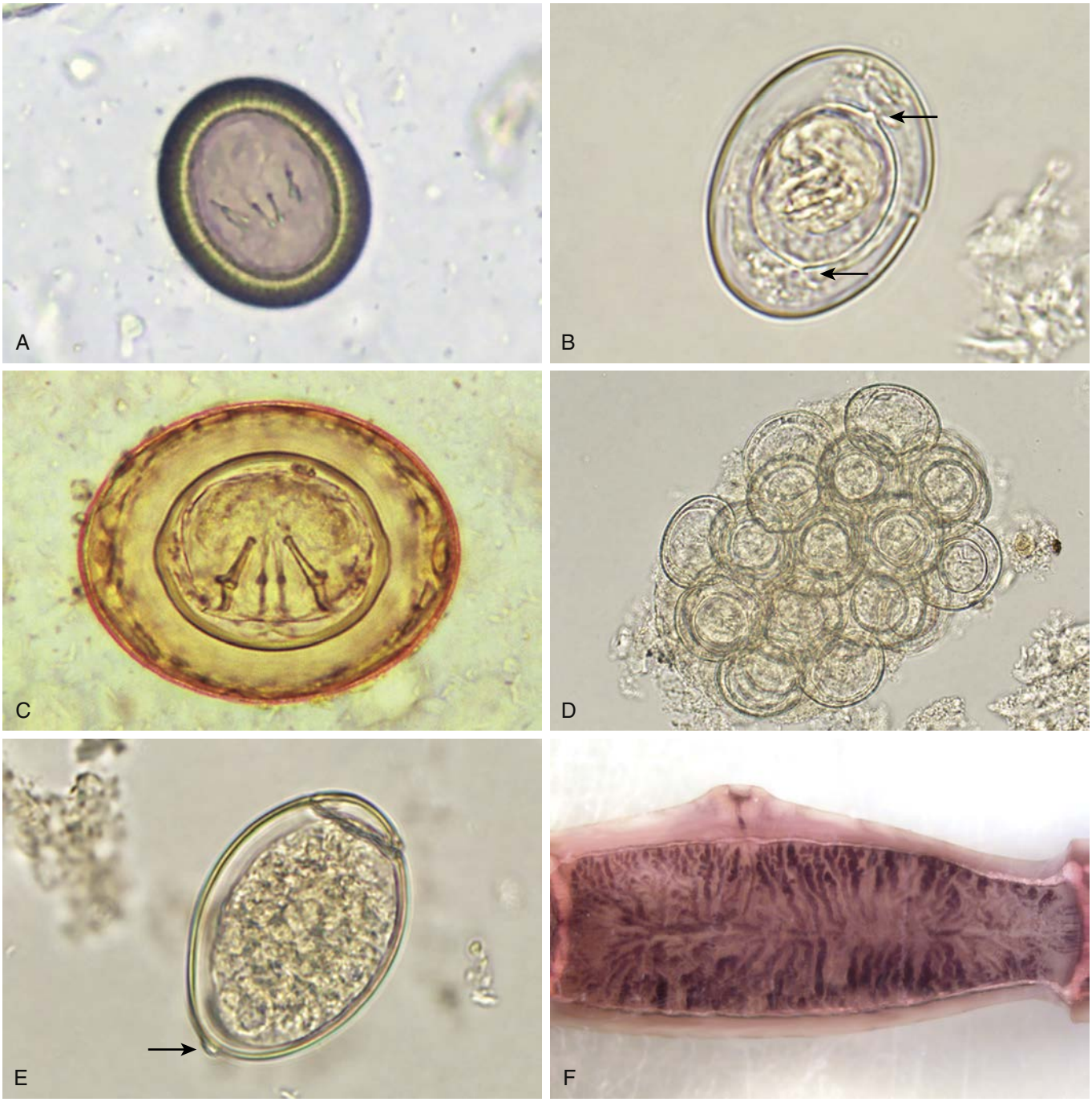

Figure 65.18 **A,** Egg of *Taenia* spp. Four of the six hooks of the oncosphere are visible (400×). **B,** Egg of *Hymenolepis nana*. Note the presence of hooklets and polar filaments *(arrows)* (400×). **C,** Egg of *Hymenolepis diminuta*. Note the lack of polar filaments. Hooks of the oncosphere are visible (iodine, 400×). **D,** Egg packet of *Dipylidium caninum* (400×). **E,** Egg of *Diphyllobothrium*/*Dibothriocephalus*/*Adenocephalus* spp. Note the operculum and small abopercular knob *(arrow)* (400×). **F,** Gravid proglottid of *T. saginata* injected through the genital pore with India ink to highlight the uterine branches (2×).

Continued

tapeworms develop in the small intestine, where they may grow to 60 cm in length. Similar to those of *H. nana*, the proglottids all have genital pores on one side; however, unlike that species, the scolex lacks an armed rostellum. Infection usually is asymptomatic because of the small number of worms likely to infect a single individual, although intestinal symptoms have occasionally been reported. Diagnosis is made by finding moderately thick-shelled, slightly ovoid, yellow-brown eggs measuring 70 to 85 µm by 60 to 80 µm in stool specimens (Fig. 65.18C). The eggs are most easily confused with those of *H. nana* but lack polar filaments.

Dibothriocephalus, Diphyllobothrium, and *Adenocephalus* (Diphyllobothriasis)

Humans may be infected by any of several species of diphyllobothriid cestodes, commonly collectively called the fish tapeworms. Historically, species implicated in human disease were placed in the genus *Diphyllobothrium*. However, the group has recently undergone taxonomic changes and most human infections are attributed to members of the genera *Dibothriocephalus* or *Adenocephalus* (Hernandez-Orts et al., 2015; Waeschenbach et al., 2017). These parasites are widely distributed in the temperate zones, especially northern Europe, Scandinavia, the former USSR, and Japan. Infection also occurs in Canada and in the north central states, the Pacific Coast states, and Alaska in the United States. Although *Dibothriocephalus latus* (formerly *Diphyllobothrium latum*) is the most common species implicated in human disease, differentiation of the infecting species cannot be made on the basis of egg morphology alone.

Diphyllobothriid cestodes inhabit the small intestine, where they can reach a length of 10 m or longer and can persist for years. Eggs are passed unembryonated in the feces and must reach a freshwater stream or lake to continue development. Following several weeks of embryonation, a ciliated larval form, the six-hooked coracidium, hatches and is ingested by a copepod, a type of microscopic crustacean. The coracidium develops into a procercoid larva, which is infective for the second intermediate host, a fish. In fish, the procercoid migrates into the tissues and develops into the plerocercoid larva. Plerocercoids may be passed up the food chain unchanged

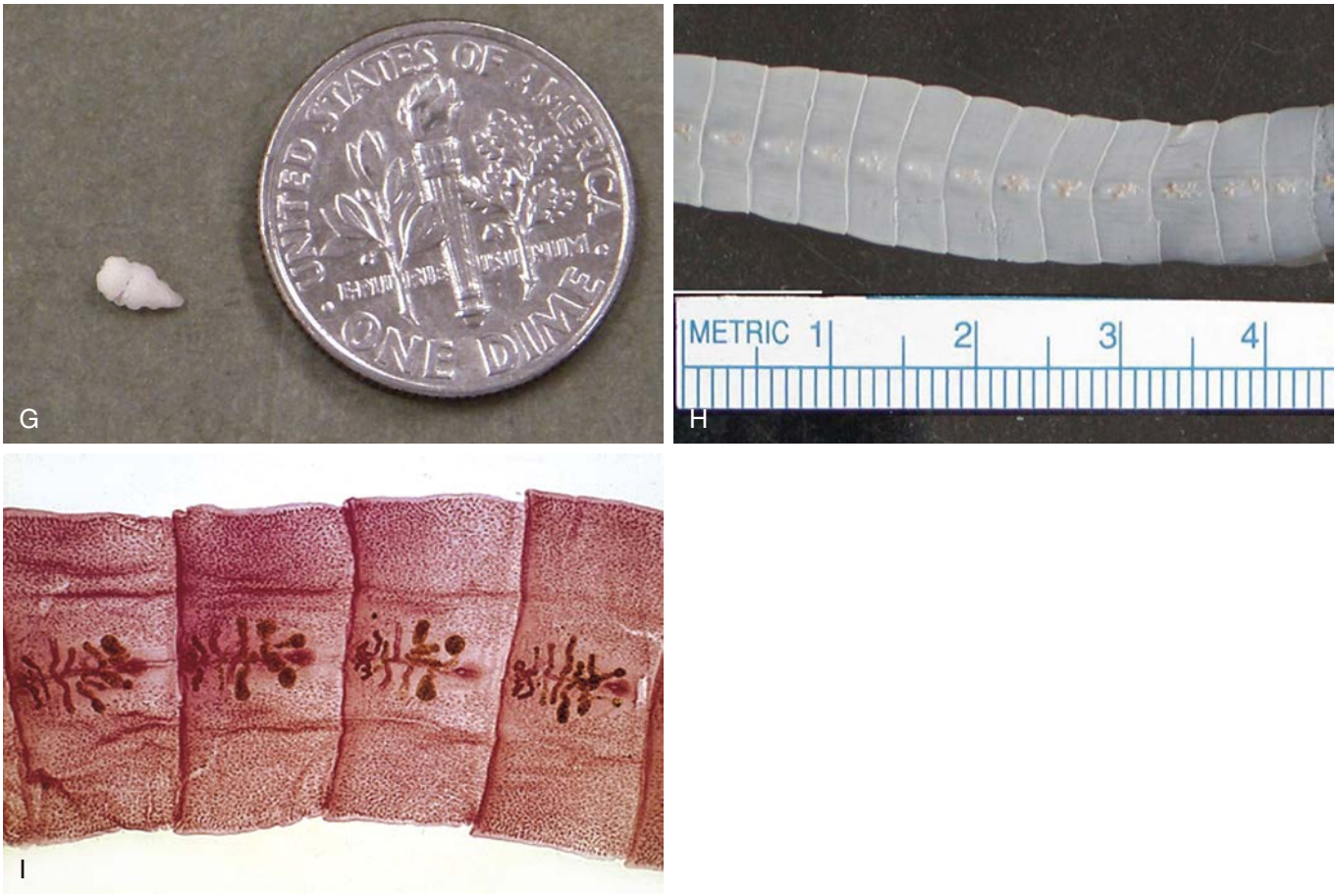

Figure 65.18—cont'd **G,** Proglottid of *Dipylidium caninum*. **H,** Proglottids of *Diphyllobothrium*/*Dibothriocephalus*/*Adenocephalus*. Note the centrally located uterus in each proglottid. **I,** Proglottids of *Dibothriocephalus* sp. (acetocarmine stain, 2×). (**G** courtesy of The College of American Pathologists Parasitology Benchtop Reference Guide.)

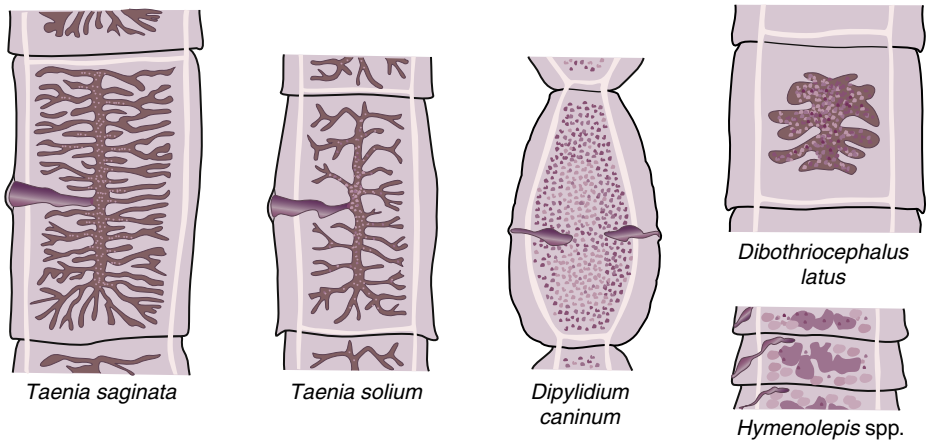

Figure 65.19 Gravid proglottids of different human tapeworms.

and accumulate in larger fish. Humans acquire these larvae through ingestion of raw or incompletely cooked fish that have spent at least part of their life in fresh water.

Adult worms mature and initiate egg production in approximately 1 month. Infection may be asymptomatic, with passage of a length of strobila being the initial complaint. In others, a variable degree of abdominal discomfort and diarrhea may be present. Rarely, intestinal obstruction occurs. In endemic areas in northern Europe, a small percentage of patients develop vitamin B_{12} deficiency and associated megaloblastic anemia due to the propensity of *Dibothriocephalus* to absorb B_{12} in the jejunum. Treatment is with praziquantel (Drugs for Parasitic Infections, 2013).

Diagnosis is made by the finding of typical yellow-brown, oval, operculate eggs (i.e., containing a lid-like opening called an operculum) in feces during O&P examination. Eggs measure 58 to 76 µm by 40 to 51 µm and, in addition to the operculum, have a small, round, knoblike projection on the abopercular end (i.e., the end opposite the operculum; Fig. 65.18E). The presence of the operculum is unique among those cestodes infecting humans. Care must be taken not to confuse these eggs with those of trematodes, especially *Paragonimus* or *Nanophyetus*, that are approximately the same size and also have an operculum. Identification of members of this complex is possible when a length of strobila (i.e., chain of proglottids) or an intact worm is passed. The scolex is rarely seen but may be recovered in the stool of patients treated with praziquantel; unlike the taeniids, it is elongate and has a pair of longitudinal grooves known as *bothria*, which replace the usual suckers of other human cestodes. Gravid proglottids are wider than they are long and have their genital pores located midventrally, adjacent to a centrally located rosette-shaped uterus (Figs. 65.18, *H*, *I*, and 65.19).

Dipylidium caninum

Dipylidium caninum is a common tapeworm of dogs and cats in most parts of the world that not infrequently infects humans, especially children.

In the usual life cycle, tapeworm eggs are ingested by flea larvae, which infest areas frequented by dogs or cats. The cysticercoid larvae persist as the flea undergoes metamorphosis to the adult stage. Accidental ingestion of the adult flea containing the infectious cysticercoid results in infection. Children are at highest risk for infection because of their close contact with pets and lack of hygiene. Worms mature in the small intestine and grow up to 70 cm in length. Infection produces few symptoms and generally causes concern only on detection of the actively moving proglottids (Cabello et al., 2011). Treatment is with praziquantel (Drugs for Parasitic Infections, 2013).

Detection is based on the finding of characteristic eggs, egg packets, or proglottids in the feces. The proglottids are rather hardy and are usually passed intact. As such, eggs or egg packets are less commonly detected in O&P examinations but can usually be expressed from proglottids in the laboratory. Spherical eggs, each containing a six-hooked embryo, measure from 24 to 40 μm in diameter and occur singly or in packets (Fig. 64.18D). The scolex is somewhat elongate, with four suckers and a small, retractable rostellum. Proglottids are barrel shaped and possess two genital pores, one on each lateral margin, which give rise to the common name *double-pored tapeworm* (see Fig. 65.19). They are commonly likened to a "grain of rice" or "cucumber seed" when seen intact in stool (Fig. 65.18G). Because they are often motile when passed, they are commonly noted by the child's caregiver.

TREMATODES

Trematodes, or flukes, are dorsoventrally flattened helminths that include both hermaphroditic forms (intestinal, liver, and lung flukes) and dioecious forms (separate sexes; seen with the blood flukes/schistosomes). Even with the hermaphroditic forms, sexual reproduction between two individual worms is the norm. All species that infect humans are characterized by the presence of an oral sucker through which the digestive tract opens and a ventral sucker (acetabulum) used for attachment. Adults vary in length from 1 mm (*Metagonimus*) to 70 mm (*Fasciola gigantica*).

Eggs reach the environment by being passed in the feces, sputum, or urine depending on the species. Hermaphroditic flukes produce operculate eggs, which may or may not be embryonated at the time of passage. Schistosome eggs are not operculated, and each contains a mature larva (miracidium) when passed. Miracidia are ciliated and are capable of penetrating the tissues of a molluscan host. Each species of trematode uses a particular species of freshwater or terrestrial snail as the first intermediate host. A complex asexual multiplication process within the snail results in the production of numerous free-swimming larvae called *cercariae*. Schistosome cercariae are capable of penetrating human skin directly, resulting in the disease schistosomiasis. Those of hermaphroditic flukes encyst on aquatic vegetation or invade the tissues of second intermediate hosts such as fish or crabs depending on the species. Ingestion of these encysted larval stages, known as *metacercariae*, results in human infection.

Human trematode infection may occur in many tropical and subtropical regions, involving considerably more species (mostly rare and sporadic intestinal infections) than can be presented here. Its presence depends on lack of adequate sewage treatment, availability of appropriate intermediate hosts, and, in the case of hermaphroditic species, dietary customs associated with ingestion of infective metacercariae in raw or undercooked plants or animals. Some of these diseases, especially schistosomiasis, expanded with the increased use of irrigation in endemic areas, which allowed for proliferation of the snail intermediate hosts. Symptoms vary depending on the number of worms parasitizing the host at a given time, the tissues and organs involved, and host responses. Many infections are asymptomatic. The treatment of choice for trematodiasis, with the exception of fascioliasis, is praziquantel (Drugs for Parasitic Infections, 2013).

The diagnosis of trematode infection is most commonly made by recovery and identification of the characteristic eggs in stool, sputum, urine, and, occasionally, tissues. Sedimentation concentration methods are most useful for recovery of these eggs. As mentioned in the prior Laboratory Methods section, zinc sulfate flotation methods are less satisfactory for detecting parasites with operculate eggs. Trematode eggs are usually shed in small numbers; thus, examination of multiple specimens is recommended for optimal detection sensitivity.

Fasciolopsis buski (Fasciolopsiasis)

This intestinal trematode is the largest species to infect humans, varying from 20 to 75 mm in length and from 8 to 20 mm in width. It occurs in many parts of China, Southeast Asia, and India and is frequently found in pigs, which serve as a natural reservoir host. Infection is acquired by ingesting infectious metacercariae on aquatic food plants such as water chestnuts and water caltrop. Worms attach to the wall of the duodenum and jejunum, where they mature to egg-laying adults in about 3 months. Symptoms such as diarrhea, epigastric pain, and nausea may develop if enough worms are present to produce ulceration of the superficial mucosa. Eosinophilia may be present, even in those who are asymptomatic. The treatment of choice for trematodiasis, with the exception of fascioliasis, is praziquantel (Drugs for Parasitic Infections, 2013).

Diagnosis is made by the finding of large (130–140 μm by 80–85 μm), yellow-brown, oval, thin-shelled eggs (Fig. 65.20C). The operculum may often be inconspicuous, and the eggs are passed unembryonated (i.e., there is no identifiable miracidium within, as is seen with schistosomes). Differentiation from *Fasciola* eggs generally is not possible, although these infections may be differentiated on the basis of geographic history and symptoms. Eggs of echinostome trematodes, which occasionally infect humans, are similar but smaller (Ash & Orihel, 2007).

Heterophyes and *Metagonimus*

These two genera include a number of species of minute (1–3 mm in length) intestinal worms that infect humans. *Heterophyes heterophyes* and *Metagonimus yokogawai* are common parasites in Asia but, along with other species, are found in other parts of the world as well. Infection is acquired by ingestion of metacercariae in raw or incompletely cooked freshwater fish. Although it is of minor medical importance, infection with these worms may produce diarrhea and abdominal pain. Infection is self-limited because the worms have a life span of only a few months. The treatment of choice for trematodiasis, with the exception of fascioliasis, is praziquantel (Drugs for Parasitic Infections, 2013).

Diagnosis is established by the finding of embryonated, operculate eggs that measure 20 to 30 μm in length by 15 to 17 μm in width. Differentiation of these eggs from those of *Clonorchis* and *Opisthorchis* may be difficult, although the operculum is more deeply seated with *Opisthorchis* and *Clonorchis* and heterophyid trematodes usually lack an abopercular knob. Such differentiation may be important for medical reasons.

Nanophyetus salmincola

Nanophyetus salmincola is a small (0.8–1.1 mm) intestinal fluke that has been reported in humans in areas of far eastern Siberia and the Pacific Northwest coast of the United States (Fritsche et al., 1989; Eastburn, 1987). These worms are acquired by ingesting raw, incompletely cooked, or home-smoked salmon or trout that contain infectious metacercariae. Symptoms are related to the number of worms present and may include abdominal pain and diarrhea, with or without eosinophilia. The treatment of choice for trematodiasis, with the exception of fascioliasis, is praziquantel (Drugs for Parasitic Infections, 2013). Eggs measuring 60 to 80 μm by 34 to 50 μm are broadly ovoid, operculate, and yellowish brown (Ash & Orihel, 2007). Thickening of the shell at the abopercular end should be differentiated from the knob seen on eggs of *Dibothriocephalus*. This fluke is the vector for a rickettsial species that produces a highly lethal infection in canines known as *salmon-poisoning disease*.

Fasciola hepatica (Fascioliasis)

Cattle, sheep, and goats in many parts of the world are infected with the liver fluke *Fasciola hepatica* and, less commonly in Africa and Asia, with the related species *Fasciola gigantica*. Adult parasites live in the biliary tree and lay eggs that are passed in the feces. Cercariae shed from the snail intermediate host encyst on aquatic vegetation, where infectious metacercariae then are available to herbivorous hosts. Humans usually acquire the infection by eating raw aquatic plants such as watercress or drinking fresh water contaminated with metacercariae. Once ingested, the larvae penetrate the intestinal wall and migrate through the peritoneal cavity to the liver. They burrow through the capsule and parenchyma, coming to reside within the bile ducts, where egg laying starts in about 2 months (Fig. 65.20F). Migration of the larvae through the liver elicits a painful inflammatory reaction both in the tissue and, later, in the bile ducts, which eventually become fibrosed. Clinical manifestations include colic, obstructive jaundice, abdominal pain and tenderness, cholelithiasis, and eosinophilia. Unlike the other trematodiasis, treatment is with triclabendazole rather than praziquantel (Drugs for Parasitic Infections, 2013).

Diagnosis is made by the finding of eggs in the stool. The unembryonated, yellow-brown, operculate eggs, 130 to 150 μm by 63 to 90 μm, cannot be distinguished from those of *Fasciolopsis* by morphology alone (see Fig. 65.20C); eggs of *F. gigantica* are typically larger, measuring 160 to 190 μm long by 70 to 90 μm wide. Spurious passage, which occurs by ingesting infected cattle or sheep liver, is diagnosed by obtaining a good history and performing a follow-up stool examination to look for elimination of the

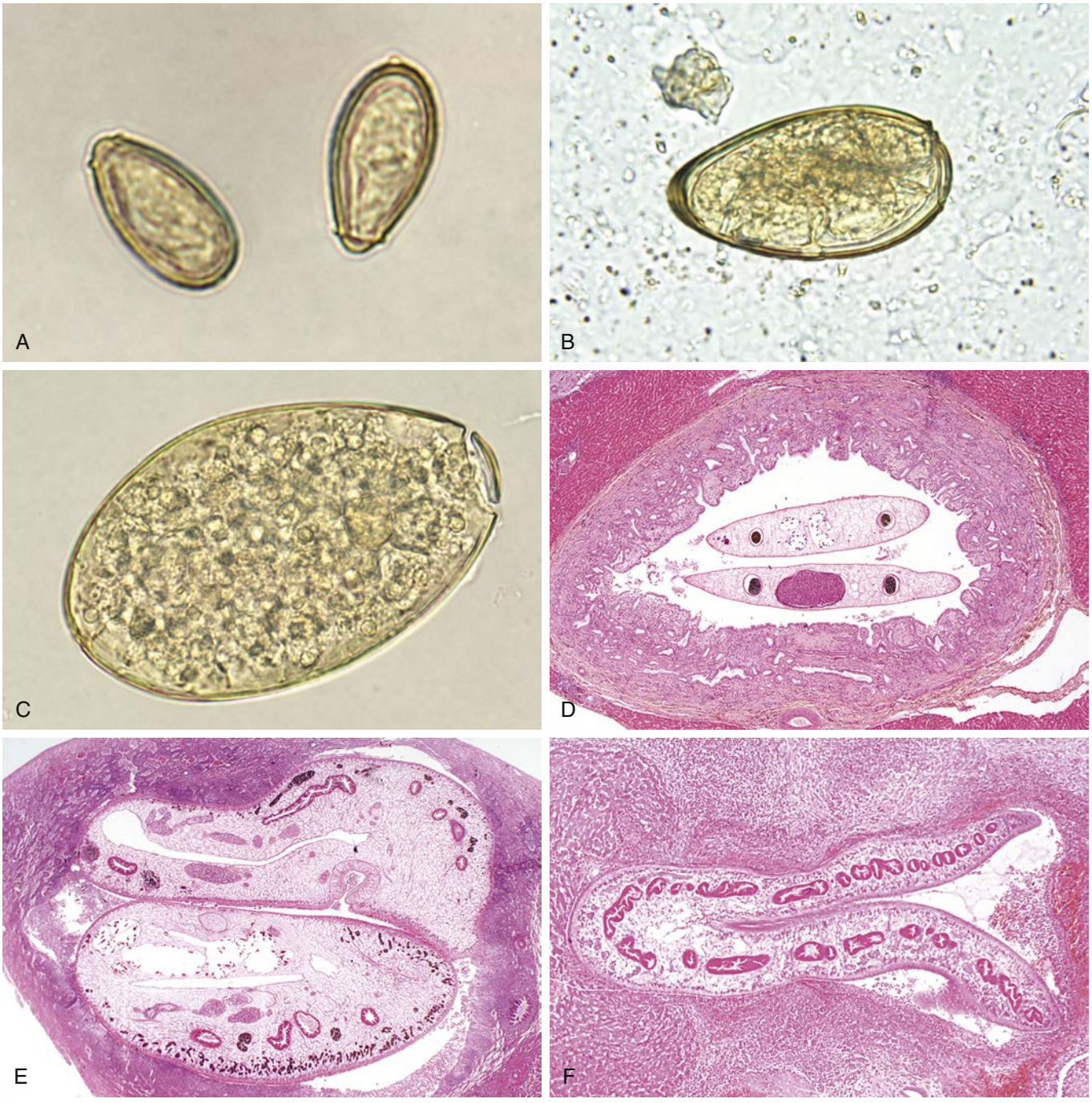

Figure 65.20 **A,** Egg of *Clonorchis sinensis* in stool. Note the prominent shouldered operculum, abopercular knob, and urn shape (1000×). **B,** Egg of *Paragonimus* sp. Note the prominent shouldered operculum and abopercular thickening (400×). **C,** Egg of *Fasciolopsis buski*, indistinguishable from that of *Fasciola hepatica*, in stool (400×). **D,** Two adult *Clonorchis sinensis* in bile duct (H&E; 40×). **E,** Pair of adult *Paragonimus* sp. in lung tissue with surrounding inflammatory reaction (H&E; 40×). **F,** Adult *Fasciola hepatica* in liver (H&E; 40×).

eggs. Serologic testing for *F. hepatica* is available in the United States at the CDC (Shin et al., 2016).

Clonorchis sinensis and *Opisthorchis* spp.

Clonorchis sinensis, the Oriental liver fluke, and a closely related species, *Opisthorchis viverrini*, inhabit the biliary system of humans and other piscivorous animals, including cats and dogs. *C. sinensis* occurs mainly in China, Taiwan, Korea, Japan, and Vietnam, whereas *O. viverrini* is found primarily in Southeast Asia, especially northern Thailand. Human infection is also known to occur with *Opisthorchis felineus* in Europe, *Amphimerus pseudofelineus* (syn. *Opisthorchis guayaquilensis*) in Ecuador, and *Metorchis conjunctus* in northern North America.

All of these parasites are acquired by the ingestion of infectious metacercariae in raw or uncooked freshwater fish. Unlike *F. hepatica*, the larvae reach the liver by migrating directly up the common duct into the liver bile ducts. Here, they can reside for 20 years and grow up to 25 mm in length (Fig. 65.20D). They produce small operculate eggs that are shed into the bile and subsequently are passed in stools.

Infection is often asymptomatic, although large numbers of flukes and repeated infection may cause inflammation of the bile ducts and subsequent hyperplasia and fibrosis (Drugs for Parasitic Infections, 2013). Of note, bile duct metaplasia and ultimate development of cholangiocarcinoma has been linked epidemiologically with long-standing infection of *Clonorchis sinensis* and *Opisthorchis* spp.

Diagnosis is made by recovering the small yellow-brown, embryonated, operculate eggs from stool (Fig. 65.20A). Eggs of *Clonorchis* cannot be readily differentiated from those of *Opisthorchis*. Both measure 25 to 35 μm by 12 to 20 μm and have a prominent, seated, or "shouldered" opercula and a small knob at the abopercular end. These eggs are difficult to differentiate from those of the *Heterophyes/Metagonimus* group, although the latter species do not have prominent, seated operculum or a small knob at the abopercular end. When specific identification is not possible, the

laboratory report should reflect this (i.e., could state, "*Clonorchis/Opisthorchis/Heterophyes/Metagonimus* eggs").

Paragonimus spp. (Paragonimiasis)

Several species of *Paragonimus* may parasitize the lungs of cats, dogs, and other carnivores, including humans. *Paragonimus westermani* and *P. heterotremus* cause human infection in many areas of Asia, whereas several species have been implicated in Central and South America, including *Paragonimus mexicanus*, *Paragonimus caliensis*, and *Paragonimus ecuadoriensis*. *Paragonimus kellicotti* has occasionally been implicated in cases from North America, and other species have been described from Africa (Ash & Orihel, 2007; Procop, 2009; Diaz, 2013).

Adult worms measure up to 12 mm by 6 mm and often are found in pairs in the lung parenchyma, where they reside encased in a host-derived fibrotic capsule (Fig. 65.20E). The capsule communicates with the bronchi, through which eggs pass to be eventually expelled in sputum or, if swallowed, the feces. A specific snail serves as the first intermediate host, while freshwater crabs and crayfish serve as second intermediates for infectious metacercariae. Humans become infected following ingestion of raw or undercooked crustaceans containing encysted metacercariae. Following ingestion, metacercariae excyst in the stomach and migrate through the intestinal wall into the peritoneal cavity, eventually reaching the lungs after penetrating the diaphragm. Maturation takes approximately 5 to 6 weeks, and worms may live for many years. Ectopic migration occurs not infrequently, resulting in infection of other organs, including the peritoneum, subcutaneous tissues, and the brain.

Symptoms, when present, may be caused by larvae migrating through tissues or by adults established in the lungs. The onset of lung infection is usually associated with fever, chills, and the appearance of eosinophilia. Once established, symptoms include chronic coughing with abundant mucus production, along with episodes of hemoptysis (Drugs for Parasitic Infections, 2013). Radiographs may show nodular shadows, calcifications, or patchy infiltrates. Eggs remaining in the lung tissues or in ectopic sites may cause an extensive granulomatous reaction.

Diagnosis is made by the finding of typical eggs in stool, sputum, or, occasionally, tissues. Eggs of the different *Paragonimus* spp. cannot be readily differentiated. Operculate, unembryonated eggs typically measure 80 to 120 μm by 45 to 70 μm and have a moderately thick, yellow-brown shell (Fig. 65.20B). The operculum is flattened and usually is set off from the rest of the shell by prominent shoulders (i.e., shouldered operculum). The abopercular end is somewhat thickened but does not have a knob. *Paragonimus* eggs may be differentiated from those of *Dibothriocephalus* and *Fasciola/Fasciolopsis*, which they superficially resemble, by size.

Schistosoma spp. (Schistosomiasis)

Schistosomiasis, or bilharzia, is among the most important parasitic diseases worldwide. As mentioned previously, this fluke is dioecious (i.e., male and female flukes are separate entities); adult flukes inhabit the veins of the mesentery or bladder; thus, they are referred to as *blood flukes*. The most important species infecting humans are *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium*, *S. intercalatum*, and *S. guineensis*. Other zoonotic species infect humans less frequently.

Adult female schistosomes are slender, measuring up to 26 mm by 0.5 mm. Males, which are slightly shorter, enfold a female using the lateral margins of the body (the gynecophoral canal) to assist in sperm transfer. When examined *in situ*, schistosomes are often found together in this arrangement (i.e., *in copula*). They are generally monogamous throughout their existence. Within the blood vessels, blood flukes elicit little or no inflammatory response. However, eggs are deposited in the smallest venule that can accommodate the female worm, eliciting a strong granulomatous host response that results in extrusion of the egg through the intestinal or bladder wall and into the lumen for excretion in stool or urine, respectively. Eggs laid in the mesenteric vessels also commonly end up in the liver. The pathology associated with schistosomiasis is primarily related to the sites of egg deposition, the numbers deposited, and the host reaction to egg antigens.

Eggs are fully embryonated when passed and readily hatch when deposited in fresh water. The miracidia penetrate an appropriate species of snail host, where they undergo transformation and extensive asexual multiplication. After about 4 weeks, large numbers of fork-tailed cercariae emerge from the infected snail. These cercariae swim actively about for hours and readily penetrate the skin of susceptible hosts, including humans. After penetration, the cercariae lose their tail to become *schistosomulae* and enter the circulation, passing through the lungs and liver, to eventually reach the mesenteric-portal vessels. A female worm will not develop to maturity without the presence of a male.

Symptoms of schistosomiasis result primarily from penetration of cercariae (cercarial dermatitis or "swimmer's itch"), from initiation of egg laying (acute schistosomiasis or Katayama fever), and as a late-stage complication of tissue proliferation and repair in response to eggs in tissues (chronic schistosomiasis). In a matter of hours after cercarial penetration, a papular rash associated with pruritus may develop. This is a sensitization phenomenon resulting from prior exposure to cercarial antigens. The most severe form of dermatitis occurs in individuals who are repeatedly exposed to cercariae of nonhuman (primarily avian) schistosomes. Cercarial dermatitis occurs worldwide and is a well-recognized condition in parts of the United States (Brant & Loker, 2009).

Initiation of egg laying by mature worms 5 to 7 weeks after infection may result in acute schistosomiasis, or Katayama fever, a serum sickness–like syndrome that occurs with heavy primary infection, especially that of *S. japonicum*. The antigenic challenge to the host is thought to result in immune complex formation (Boros, 1989).

Chronic infection results in continued deposition of eggs, many of which remain in the body. Granulomas produced around these eggs in the intestine and in the bladder are gradually replaced by collagen, resulting in fibrosis and scarring. Eggs trapped in the liver may induce pipe-stem fibrosis with obstruction to portal blood flow. Occasionally, eggs are deposited in ectopic sites, such as the spinal cord, lungs, or brain (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018). Treatment is with praziquantel, which will kill the adult worms and prevent further damage (Drugs for Parasitic Infections, 2013). However, it will not reverse the scarring that previously occurred due to egg deposition.

Diagnosis is established by demonstrating eggs in feces or urine by direct wet mount or sedimentation-based concentration methods. Flotation concentration is not satisfactory for recovery of heavy schistosome eggs. Eggs also may be detected in biopsies of rectal, bladder, and, occasionally, liver tissues by crush preparation or in histologic sections (Fig. 65.21).

Serologic tests may be helpful in screening persons who have traveled to endemic areas and those with negative urine or stool examination who are at risk for infection, although it cannot be reliably correlated with worm burden, clinical status, egg production, or prognosis. Although not widely available, a limited number of reference laboratories and the CDC provide testing. Generally, serologic testing varies with the antigens used and test methods employed. The CDC uses the Falcon assay screening test in a kinetic enzyme-linked immunosorbent assay (FAST-ELISA). Sera that are positive by the screening test are further evaluated by immunoblot to improve specificity (Tsang & Wilkins, 1997).

Schistosoma mansoni

Schistosoma mansoni occurs in Africa, especially in the tropical areas and the Nile delta, southern Africa, and Madagascar, Brazil, Venezuela, Surinam, and certain Caribbean islands, including Puerto Rico. Adult *S. mansoni* live primarily in the portal vein and in the distribution of the inferior mesenteric vein. Initial deposition of eggs in the large intestine may produce abdominal pain and dysentery, with abundant blood and mucus in the stool. Eggs may be detected in feces at this time. Chronic infection may result in liver fibrosis and portal hypertension depending on the number of worms present; eggs may be more difficult to find in feces during this stage.

Eggs measure 116 to 180 μm by 45 to 58 μm, are oval, and possess a large distinctive lateral spine that protrudes from the side of the egg near one end (Figs. 65.21A and 65.21B). If the spine is not visible, the egg may be rotated by gently tapping the coverslip. Movement of the miracidium within the egg may be evident in unfixed material and, when seen, indicates active infection. Concentration techniques may be required to detect eggs because individuals with limited exposure or with chronic infection may pass few of them.

Schistosoma japonicum

Schistosoma japonicum, which occurs in China, southeast Asia, and the Philippines, causes disease that is clinically similar to that of *S. mansoni* but often more serious because many more (up to 10 times as many) eggs are produced by *S. japonicum*. The disease has been essentially eliminated from Japan, although animal reservoirs still exist. Adult worms live primarily in the distribution of the superior mesenteric vein, and eggs readily reach the liver, inducing fibrosis and portal hypertension as a common complication of chronic infection. The smaller size of the eggs is thought to predispose them to ectopic dissemination, especially to the brain and spinal cord. The eggs are broadly oval, measuring 75 to 90 μm by 60 to 68 μm, and have an inconspicuous lateral spine, which may be difficult to demonstrate (see Figs. 65.21C and 65.21D).

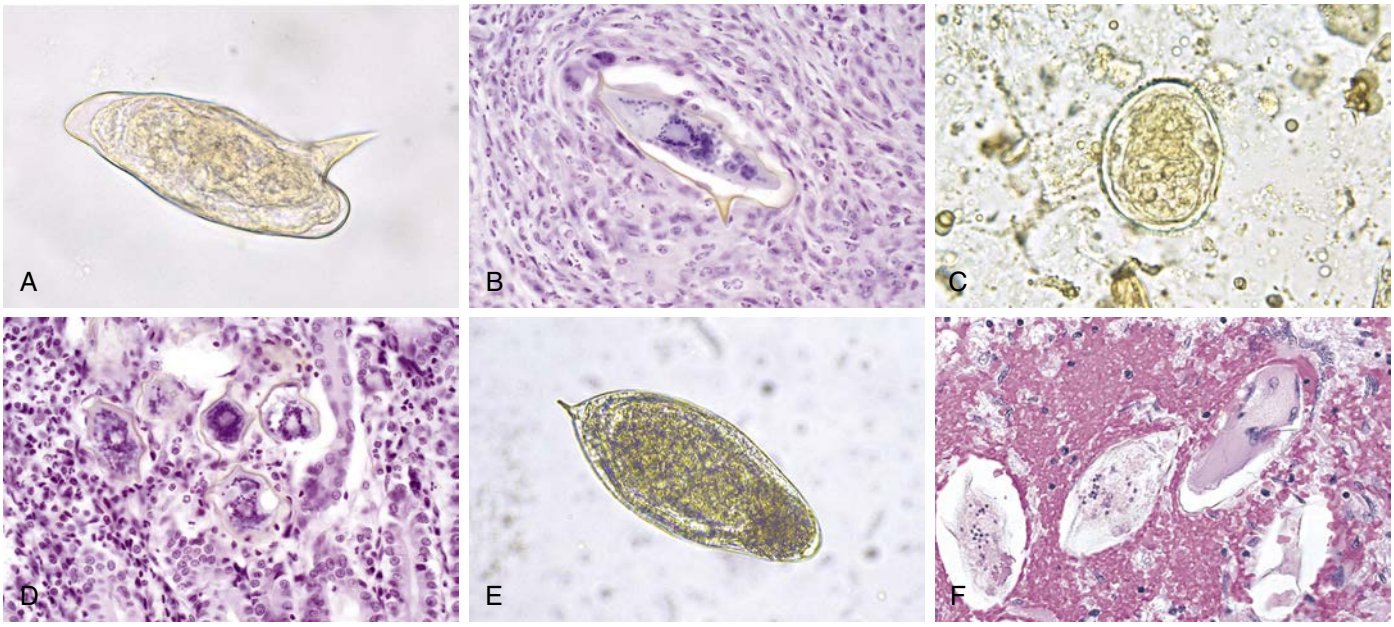

Figure 65.21 **A,** Egg of *Schistosoma mansoni*. Note the prominent lateral spine (400×). **B,** Egg of *S. mansoni* in an intestinal biopsy. Note the presence of a lateral spine (H&E; 400×). **C,** Egg of *Schistosoma japonicum*. Note the presence of a rudimentary lateral spine (400×). **D,** Eggs of *S. japonicum* in a small-bowel biopsy (H&E; 200×). **E,** Egg of *Schistosoma haematobium* found in urine sediment. Note the presence of a terminal spine (400×). **F,** Eggs of *S. haematobium* seen in a vulvar granuloma. Note the presence of a terminal spine (H&E; 400×).

Schistosoma mekongi

This species occurs in humans and animal reservoirs in countries along the Mekong River, especially Cambodia and Laos (Ash & Orihel, 2007). It is similar to *S. japonicum* but is differentiated from that species by several biological characteristics and by smaller eggs (60–70 μm by 52–61 μm), which otherwise are indistinguishable from those of *S. japonicum*.

Schistosoma haematobium

Urinary schistosomiasis occurs in many parts of Africa, the Middle East, and Madagascar, and has been introduced to Corsica, Italy (Ramalli et al., 2018). Parasites migrate via the hemorrhoidal veins to the venous plexuses of the urinary bladder, prostate, uterus, and vagina. One of the earliest and most common symptoms of infection is hematuria, especially at the end of micturition. Chronic infection may cause pelvic pain and bladder colic, with an increased desire to urinate. Accumulation of eggs in the tissues may result in hypertrophy of the urothelium, squamous metaplasia, and marked fibrosis, which may progress to obstruction and, ultimately, renal failure. Urinary schistosomiasis also has been associated with squamous cell carcinoma of the bladder (Meyers, 2000).

Eggs can be recovered from the urine by examination of spun sediment or through filtration. They are elongate, measuring 112 to 180 μm by 40 to 70 μm, and have a characteristic terminal spine (see Figs. 65.21E and 65.21F).

Schistosoma intercalatum and *S. guineensis*

These species occur in parts of central and western Africa and produce intestinal schistosomiasis. *Schistosoma guineensis* (formerly the "Lower Guinea strain" of *S. intercalatum*) occurs generally in central and western Africa while *S. intercalatum* proper (formerly the Zaire strain) is endemic to the Democratic Republic of the Congo. Eggs have a terminal spine, resembling those of *S. haematobium*, but they occur primarily in the feces and are larger (140–240 μm by 50–85 μm) (Webster et al., 2006).

TISSUE HELMINTHS

NEMATODES

Filarial Nematodes (Filariasis)

Filarial nematodes are arthropod-transmitted parasites of vertebrate animals. Adult male and female worms are long and slender, and are known to inhabit a variety of tissues, including subcutaneous tissues, lymphatics, blood vessels, peritoneal and pleural cavities, and the heart. All species produce larvae known as *microfilariae*, which may be recovered from blood or skin, depending on the species. The microfilariae of some species circulate in the blood with a well-defined periodicity (diurnal or nocturnal), whereas others do not. Microfilariae continue their development only in the appropriate arthropod vector, usually a mosquito or other fly, where they mature to the infective stage. Such larvae then are deposited in the tissues of a definitive host when the vector takes another blood meal.

The diagnosis of filariasis usually is made by the finding of microfilariae in the blood or skin because adult stages are often sequestered in the tissues. Use of Giemsa or hematoxylin-stained thick and thin smears of peripheral blood is routine. However, more sensitive procedures—such as membrane filter, Knott concentration, or saponin lysis—may also be required for detection (Ash & Orihel, 2007; Mathison et al., 2019). Microfilariae may also be seen moving in direct mounts of blood or tissue fluid.

Identification of the infecting filarial worm is important for directing therapy since it varies among the common human pathogens. Principal characteristics used for identification of microfilariae include the presence or absence of a sheath and its staining characteristics, the shape of the tail and the distribution of cell nuclei within, and the size of the cephalic space and appearance of its nuclear column (see organism-specific details in the following sections). An algorithm for identifying the common human-infecting microfilariae has recently been published (Mathison et al., 2019). Because microfilariae of *Wuchereria bancrofti* and *Brugia* spp. usually display a nocturnal periodicity, blood from patients suspected to be infected with these organisms should be drawn between the hours of 10 PM and 2 AM. *Loa* displays diurnal periodicity; thus, blood preferably should be drawn between 10:00 AM and 2 PM. *Mansonella ozzardi* and *M. perstans* are intrinsically nonperiodic; thus, blood can be obtained at any time for detection of these species. In contrast to the other filarial, the microfilariae of *Mansonella streptocerca* and *Onchocerca volvulus* are present in the skin and are detected by examination of skin snips or punch biopsies rather than blood films. Microfilariae in the skin do not exhibit periodicity.

Serologic tests for the diagnosis of lymphatic filariasis may prove helpful in select patients, especially those who are not native to endemic areas. Such methods are limited in their ability to distinguish between past exposure and current infection, however. Consequently, infection with other nematode species may result in the appearance of cross-reacting antibodies, with resultant false-positive results. Antigen detection tests also may be of value in the diagnosis of lymphatic filariasis but generally are not available in nonendemic settings and, to date, are not FDA approved for clinical diagnosis in the United States (Wilkins & Nutman, 2015; Norice-Tra & Nutman, 2019).

Wuchereria bancrofti (Lymphatic Filariasis)

This species, responsible for Bancroftian filariasis, is the most common filarial species to infect humans. Endemic areas include central and northern Africa, India, Southeast Asia, certain South Pacific islands, and portions of Central and South America and the West Indies. Adult worms reside in the lymphatic system, where chronic infection and reinfection result in lymphadenopathy and lymphangitis, which may progress to lymphedema

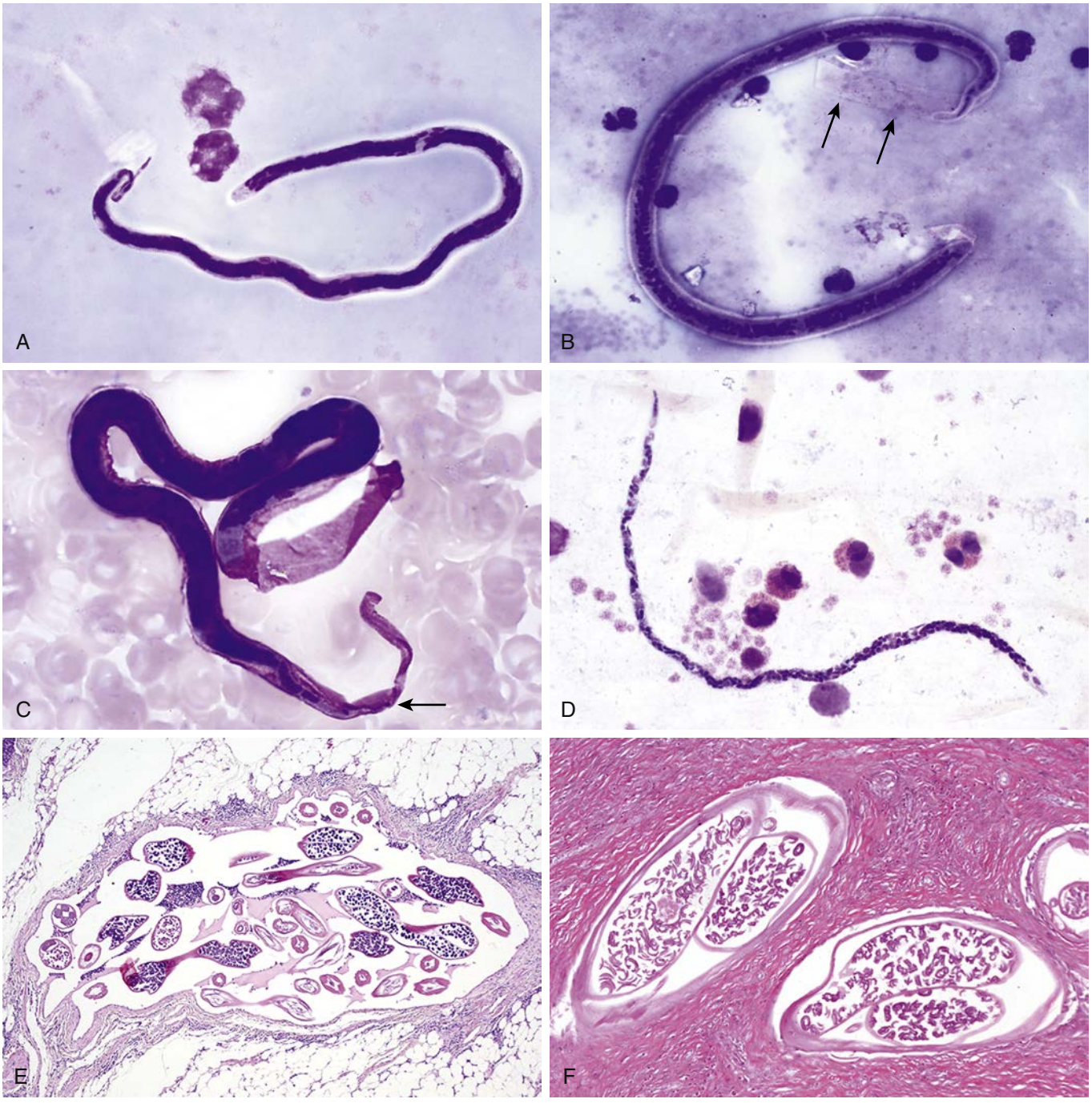

Figure 65.22 **A,** Sheathed microfilaria of *Loa*; cell nuclei extend to the tail tip. Note that the sheath is seen by its negative staining (Giemsa; 1000×). **B,** Sheathed microfilaria of *W. bancrofti*; cell nuclei do not extend to the tip of the tail. Note that the sheath *(arrows)* does not readily stain with Giemsa (1000×). **C,** Sheathed microfilaria of *Brugia malayi*; two solitary cell nuclei are seen in the tail tip. Note the deep pink–staining sheath (Giemsa; 1000×). **D,** Unsheathed microfilaria of *Mansonella perstans*; cell nuclei extend to the tail tip (Giemsa; 1000×). **E,** *Wuchereria bancrofti*, cross-section of adults in lymphatics. Note the associated fibrosis of the lymph vessel (H&E; 20×). **F,** *Onchocerca volvulus*, cross-section of adult in skin nodule, showing a classic "double-barrel" uterus (H&E; 100×).

and obstructive fibrosis (Fig. 65.22E). Severe involvement of the lower extremities and genitalia may result in a condition of enlarged limbs or scrotum referred to as *elephantiasis*. Diethylcarbamazine (DEC) is the drug of choice for *W. bancrofti* infection (Drugs for Parasitic Infections, 2013), but supportive care for irreversible edema and fibrosis may also be indicated.

In most areas, microfilariae circulate in peripheral blood with a nocturnal periodicity that corresponds with feeding activities of the usual vectors—*Culex*, *Aedes*, and *Anopheles* mosquitoes. Of note, infections originating in the South Pacific may be without periodicity, or subperiodic. The microfilariae are sheathed, although this may not always be obvious with Giemsa staining, in which the sheath may appear only as a negative outline. However, a hematoxylin stain will stain the sheath and allow for its ready identification (Mathison et al., 2019). The tail is pointed, and no nuclei are present in the tip. The cephalic space is not as long as it is wide, and the nuclei in the nuclear column are distinct (Figs. 65.22B and 65.23). Concentration procedures may be necessary for recovery because microfilariae may be present in small numbers.

Brugia malayi (Lymphatic Filariasis)

This species produces disease similar to that of *W. bancrofti*, although it is often milder and more frequently involves the lymphatics of the upper extremities. DEC is the drug of choice for infection (Drugs for Parasitic Infections, 2013), but supportive care for irreversible edema and fibrosis may also be indicated. The parasite occurs mainly in India, Southeast Asia, Korea, the Philippines, and Japan. Of note, human infection with related zoonotic *Brugia* spp. is encountered periodically in the Americas but is typically diagnosed by the finding of immature worms in biopsy specimens (Orihel & Ash, 2007). Surgical removal is curative for zoonotic *Brugia* infections.

The microfilariae circulate in the blood and are primarily nocturnally periodic. Microfilarial sheaths of *Brugia malayi* stain well with Giemsa

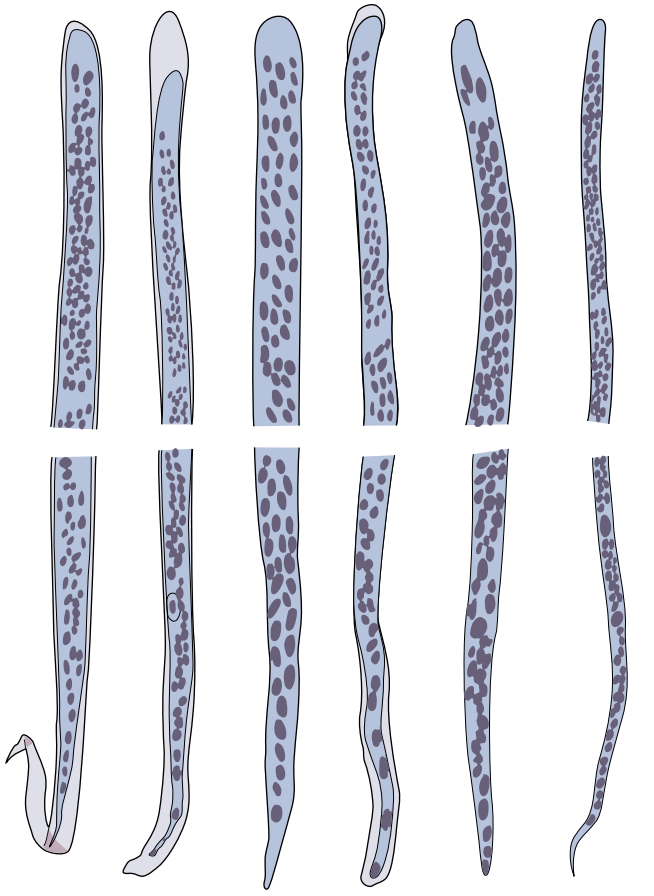

Figure 65.23 Anterior and posterior ends of microfilariae most commonly found in humans. All camera lucida drawings.

stain, often appearing bright pink. However, it should be noted that this phenomenon is not exclusive to *B. malayi* and may not be seen in some cases. Therefore, sheath staining should not be used as a sole diagnostic feature for *B. malayi*. The tail has a swelling at the tip and has two solitary nuclei located beyond the ends of the nuclear column separated by appreciable gaps (termed *subterminal* and *terminal* nuclei). The cephalic space may be much longer than it is wide (Figs. 65.22C and 65.23). *Brugia timori* is a distinct species occurring restricted to several islands in the Lesser Sunda archipelago, including the islands of Timor, Sumba, Lembata, Panta, and Alor. Microfilariae are very similar to those of *B. malayi*, although somewhat larger, and the sheath typically does not stain bright pink with Giemsa.

Loa (Loiasis)

Known as the African eye worm, the adult *Loa loa* lives in subcutaneous tissues. The nematodes migrate continuously, producing transient (2–3 days) local inflammatory reactions known as *Calabar* or *fugitive swellings*. Their occasional appearance in the conjunctiva allows them to be recognized and surgically excised. The presence of *L. loa* adults in the conjunctiva may cause a local inflammatory response but does not lead to blindness (compared with *O. volvulus*; see later discussion). DEC is the drug of choice, but caution must be used in cases of high microfilaremia (≥8000 microfilaria/mL) since rapid killing of circulating microfilariae may cause encephalitis. Upfront treatment with albendazole or apheresis is useful for reducing microfilaremia prior to DEC therapy (Drugs for Parasitic Infections, 2013). Loiasis occurs primarily in west and central Africa, where deer flies of the genus *Chrysops* serve as the vector. This parasite elicits strong eosinophilia.

Loiasis occasionally has been seen in the United States and other nonendemic settings in immigrants and travelers. In these settings, adult filarial nematodes recovered from the eye must be differentiated from *Dirofilaria* species that have a similar morphologic appearance. This can be accomplished by examining the cuticle of the retrieved worm. Adults of *L. loa* have random pimple-like cuticular projections called *bosses*, while *Dirofilaria* spp. have longitudinal cuticular ridges. The microfilariae, which circulate in the blood with diurnal periodicity, are sheathed. However, like the microfilariae of *W. bancrofti* and *B. timori*, the sheath does not stain with Giemsa stain. As with the others, a hematoxylin stain can be used to highlight the presence of the sheath. Nuclei in the tail extend to the rounded tip. The nuclear column is usually compact, and the cephalic space is short (Figs. 65.22A and 65.23) (Ash & Orihel, 2007; Mathison et al., 2019).

Onchocerca volvulus (Onchocerciasis)

Onchocerciasis due to *O. volvulus* is a leading cause of blindness in endemic areas, which include central Africa, Central America (Mexico and Guatemala), and northern South America. Vectors are black flies of the genus *Simulium*. Adult worms live in hard, fibrous nodules (onchocercomas) in subcutaneous and deeper tissues that can grow to be 40 mm in diameter (Fig. 65.22F). Nodules tend to occur on the upper half of the body in patients from Central America and on the lower half in those from Africa. Adult worms produce microfilariae that migrate continuously throughout the skin. Complications arise from the migratory activities of microfilariae, resulting in several forms of dermatitis. Movement of microfilariae through the surface of the eye may result in keratitis, corneal opacity, and damage to the anterior and posterior chambers and iris, leading to blindness with repeated infection over time. The drug of choice is ivermectin, which kills the circulating microfilariae and limits the morbidity associated with their migration. Unlike the other filariae, DEC should not be used for onchocerciasis since the rapid killing of microfilariae in the eye may lead to irreversible blindness (Drugs for Parasitic Infections, 2013). Ivermectin will not kill the adult *O. volvulus*; however, doxycycline may be coadministered with ivermectin to kill the obligate commensal bacterium, *Wolbachia*, found in the adult worms, and thus eliminate infection (Drugs for Parasitic Infections, 2013). Surgical removal of the onchocercoma may also be performed to eliminate individual infection.

In the United States, Europe, and northern Asia, human infection with the zoonotic species *O. lupi* can occur. Reported human cases in the Old World usually manifested with ocular involvement while cases in the United States involved periorbital, cervical, and subcutaneous nodules (Cantey et al., 2016). Removal is curative.

Diagnosis of *O. volvulus* infection is made by the finding of typical microfilariae in teased skin snips or skin biopsies, preferably taken from over the scapular region or from the iliac crest, when placed in saline. Alternatively, fluids expressed from scarified skin or aspirates of nodules may be examined (Garcia, 2016). Microfilariae in stained preparations lack both a sheath and nuclei in the tail tip (see Fig. 65.23). Detection of zoonotic infections with *O. lupi* is typically via histopathologic examination and identification of the adult worm in excised nodules (Cantey et al., 2016).

Mansonella spp.

Several species of *Mansonella* infect humans, but all are generally regarded as causing little pathology and must be differentiated from the truly pathogenic filarial species discussed previously. *Mansonella ozzardi* is found in Central and South America and in some areas of the Caribbean. Adult parasites reside in subcutaneous tissues. *Mansonella perstans* occurs in many areas of tropical Africa and sporadically in Central and South America and the Caribbean. Adults are thought to reside primarily in body cavities and the mesenteries. DEC is ineffective against *M. perstans* and *M. ozzardi*, but doxycycline may be useful for strains that contain *Wolbachia* as an obligate commensal bacterium (Drugs for Parasitic Infections, 2013). Microfilariae of both species are unsheathed and circulate in peripheral blood without evidence of periodicity. *M. ozzardi* microfilariae have a thin, pointed tail without nuclei, whereas the tail of *M. perstans* is broad and blunt with nuclei extending to the tip (Figs. 65.22D and 65.23). *Mansonella streptocerca*, which is found in tropical Africa, may be confused with *Onchocerca volvulus* because both adult and microfilarial stages occur in skin and subcutaneous tissues. Also, dermatitis may be produced by this species. DEC is the drug of choice (Drugs for Parasitic Infections, 2013). Microfilariae of this species, which may be recovered in skin snips, are unsheathed and have a crook in the tail with nuclei extending to the tip. All species of *Mansonella* are transmitted by midges (gnats) of the genus *Culicoides*, although *M. ozzardi* can also be transmitted by black flies of the genus *Simulium*.

Zoonotic Filarial Nematodes

Certain filarial nematodes of the genera *Dirofilaria*, *Brugia*, and *Onchocerca* that naturally parasitize wild and domestic mammals sporadically infect humans. Zoonotic infections with *Brugia* and *Onchocerca* species were discussed earlier. *Dirofilaria immitis*, commonly known as the canine heartworm, is widely distributed, and human infection is well documented. The mosquito-transmitted larval stage migrates to the right side of the heart. When the worm dies, it is swept into a small pulmonary artery, producing

a granulomatous nodule that appears as a coin lesion on a chest radiograph. Confirmatory diagnosis usually is made by histologic examination of the nodule following a positive chest radiograph (Pritt, 2018). Infected individuals are usually asymptomatic and disease is detected incidentally on radiologic imaging for some other indication. When present, symptoms include self-limited dyspnea, chest pain, fever, hemoptysis, and malaise. Treatment is not generally indicated.

Other species of *Dirofilaria*—including *D. tenuis*, *D. repens*, *D. ursi*, *D. striata*, and *D. subdermata*—commonly cause subcutaneous nodules in humans but usually fail to produce microfilariae. Such nodules have been reported from many body sites, including the face, conjunctiva, and breast, and usually are removed surgically. *D. tenuis* (southeastern United States) and *D. repens* (Europe and Asia) may also have ocular involvement and are diagnosed by the recovery of intact adult worms from the conjunctiva. Histologic examination often reveals a prominent mixed inflammatory reaction surrounding a dead worm. Criteria for identification of zoonotic filarial nematodes in tissue sections may be found elsewhere (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018). Surgical removal is curative.

OTHER

Dracunculus medinensis (Dracunculiasis)

Adults of *Dracunculus medinensis*, otherwise known as the Guinea worm, live in subcutaneous tissues and become clinically evident when the female worm migrates to the skin surface and produces a blister, usually on the lower extremities. When the extremity is immersed in water, the blister ruptures, releasing swarms of motile first-stage (L1) larvae from the female worm into the water. Freshwater copepods ingest the larvae, which then mature to the infective stage (L3 larvae) and are transmitted back to humans when copepods are accidentally swallowed in drinking water.

The disease was previously widespread, being found in areas of Africa, the Middle East, and Asia. Extensive control efforts have been made in recent years to eradicate this destructive parasite and success is thought to be close at hand, although the recent discovery of infected dogs in Chad, Ethiopia, and Mali has led to concerns that dogs may serve as an important disease reservoir (Eberhard et al., 2016; Hopkins et al., 2018). While not typically life-threatening, infection can result in disfiguring cutaneous scars and more serious secondary bacterial infection.

Diagnosis is made by identifying the female worm emerging at the skin surface with larvae in the discharge fluid. The worm is usually removed by gently pulling it out over a period of days, while winding it around a stick. Care must be taken not to damage it during removal. The long length of the worm (70–120 cm) generally precludes its surgical removal. Should the worm die in situ, pronounced inflammatory reaction along the length of the worm in its subcutaneous location and secondary bacterial infection may disable the affected individual.

Angiostrongylus cantonensis and *Angiostrongylus costaricensis* (Angiostrongyliasis)

Human eosinophilic meningoencephalitis, caused by *Angiostrongylus cantonensis*, occurs both in epidemics and sporadically in many areas of the South Pacific, Southeast Asia, and Taiwan. As the epidemiology becomes better understood, other cases have been reported from Australia, Cuba, Ecuador, Brazil, Puerto Rico, Madagascar, and mainland Africa. Human infection is rare in the continental United States. However, the disease has become problematic in Hawaii, where rats and terrestrial mollusks (snails, slugs, and invasive semi-slugs) have become serious pests. The mature parasite normally is found in the pulmonary arteries of rats. Larvae migrate up the trachea and are passed in the feces. They develop to the infective stage in slugs or land snails and, when eaten by the usual rodent host, migrate through the brain before maturing in the pulmonary arteries. Humans acquire the infection by eating large edible snails; raw or incompletely cooked shrimp or crabs, which may serve as paratenic hosts; or vegetables contaminated with infected mollusks. In humans, *A. cantonensis* larvae migrate to the CNS, producing generally nonfatal meningitis with high spinal fluid eosinophilia. Humans are a dead-end host for *A. cantonensis*; thus, further development does not occur. Neurologic symptoms begin with the death and inflammatory response to dying worms in the brain (Fig. 65.16L); the severity of symptoms is directly linked to worm burden. Treatment is not well defined; albendazole or mebendazole with or without corticosteroids may shorten the course of infection (Drugs for Parasitic Infections, 2013). Diagnosis is established both clinically and historically, although larvae occasionally have been recovered from spinal fluid (da Silva & Mathison, 2018). PCR is available at the CDC and the Hawaii Department of Public Health. Serologic tests are available in Asia but are not thought to be useful for clinical diagnosis, as there is high cross-reactivity with other nematode infections and high seroprevalence rates in asymptomatic individuals living in endemic settings have been reported.

Angiostrongylus costaricensis occurs widely in Central and South America (da Silva & Mathison, 2018). This parasite, which is responsible for the intestinal form of angiostrongyliasis, normally resides in the mesenteric arteries of the ileum and cecum of rodents. Human infection occurs in the same anatomic location but often results in granulomatous inflammation and symptoms of acute abdomen. Infection is self-limited, and treatment is not well defined (Drugs for Parasitic Infections, 2013). Diagnosis is made by histologic examination of surgical specimens and the finding of adults or eggs in the tissues (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018).

Trichinella species (Trichinosis or Trichinellosis)

Human trichinellosis occurs worldwide, although its incidence in the United States has been in steady decline, with fewer than 25 cases reported each year due to improved animal husbandry practices. Humans acquire the infection through ingestion of raw or incompletely cooked meat that contains infective larvae. Common sources of human infection in the United States are wild boar, bear, and occasionally walrus or domestic pigs raised on small, nonregulated personal or community farms (Mathison & Pritt, 2018). Ingested first-stage larvae mature in the small intestine, where gravid females produce new larvae for 2 to 3 weeks. During this stage, gastrointestinal symptoms occur, lasting several days. Larvae subsequently enter lymphatics and venules of the intestine, thus reaching the general circulation. They primarily invade the skeletal musculature, where they undergo further development and encapsulation. During the migratory and encapsulation phases, fever, muscle pain, respiratory difficulties, periorbital edema, and eosinophilia may develop, depending on the inoculating dose. During this stage of infection, treatment with albendazole and steroids may be indicated (Drugs for Parasitic Infections, 2013). After the parasites have encysted, few symptoms are noted. Encysted larvae may remain viable for several years, although they eventually become calcified. Perpetuation of the life cycle requires continuous cycles of carnivores and humans are typically a dead-end host.

Diagnosis is usually made on the basis of epidemiologic history and clinical symptoms and is confirmed by antibody detection by EIA (Wilkins & Nutman, 2015). *Trichinella* larvae may be observed in skeletal muscle biopsy specimens, particularly the gastrocnemius or deltoid muscles (see Fig. 65.16H), but the sensitivity is low.

Larval Migrans

Larval migrans is caused by prolonged wandering through body tissues of larvae of certain hookworms, ascarids, and *Strongyloides* species that normally infect wild or domestic animals. The syndrome varies with the species involved, the number of worms, and the tissues parasitized.

Cutaneous larva migrans, or ground itch, is produced by the cutaneous wanderings of cat or dog hookworms of the genus *Ancylostoma*, which penetrate the skin but cannot mature in the usual pattern. Serpiginous, erythematous, and pruritic tracks are apparent on the skin in areas where there has been contact with the ground. This is particularly problematic in warmer, humid climates, where eggs and larvae of these hookworms survive longer. Some species of *Strongyloides* that parasitize wild animals may cause a similar dermatitis (Beaver et al., 1984). Treatment is with albendazole (Drugs for Parasitic Infections, 2013).

Visceral larva migrans (VLM) is produced primarily by the random wanderings of the dog ascarid *Toxocara canis* and, to a lesser degree, by *Toxocara cati* from the domestic cat and *Baylisascaris procyonis* from the raccoon. Toxocariasis is one of 5 NPIs targeted by the CDC for public health action (CDC, 2018b). Children are usually infected following the accidental or intentional ingestion of eggs in soil contaminated with dog, cat, or raccoon feces. After hatching, the larvae are unable to complete their usual cycle and instead begin a prolonged migration through various tissues and organs. Children may present with failure to thrive and may display fever, hepatomegaly, pneumonitis, hypereosinophilia, and hypergammaglobulinemia. An inflammatory reaction in the retina from ocular larva migrans (OLM) may mimic retinoblastoma, a malignant tumor from which it must be differentiated. VLM is generally treated using albendazole or mebendazole. OLM is more difficult to treat due to the potential for ocular damage and may include surgical removal of the migrating larva and corticosteroids to decrease inflammation (Drugs for Parasitic Infections, 2013; CDC, 2018b).

Diagnosis of VLM is usually made on clinical grounds because the parasite is rarely recovered. Serologic tests may be helpful in confirming a presumptive diagnosis. The currently recommended procedure is an EIA that uses larval-stage excretory-secretory antigens (Rascoe et al., 2013; Wilkins, 2014).

A VLM-like syndrome may also be caused by species of *Gnathostoma* that infect the stomach of various mammals. Human infection is most common in Southeast Asia but has been reported in Mexico and Ecuador. These parasites use a copepod for the first intermediate host and fish and amphibians as secondary hosts. A variety of reptiles, birds, and mammals may serve as paratenic hosts. The larvae may migrate through subcutaneous tissues, causing transient swellings, and to deeper tissues, eventually invading the CNS. Treatment is with albendazole or ivermectin, with surgical removal of the migrating larva if possible (Drugs for Parasitic Infections, 2013). The occurrence of migratory lesions and a history of eating raw fish may be helpful in establishing a clinical diagnosis (Orihel & Ash, 1995).

Capillaria hepatica (Hepatic Capillariasis)

Although normally a parasite common to rodents, this species occasionally causes human disease, especially in children, in whom it may mimic VLM, hepatitis, amebic liver abscess, and other diseases. In the usual rodent host, eggs are ingested and resulting larvae migrate to the liver, where they mature and deposit eggs directly in the parenchyma. When the liver is eaten by a predator, the eggs are passed out in the feces and contaminate soil. Eggs may also be liberated into the environment by the natural death and decay of the rodent host. Children are at particular risk for acquiring the eggs if they eat dirt (Li et al., 2010). Diagnosis is usually made by examination of liver biopsies or tissue obtained at autopsy. Eggs are readily recognized in tissue biopsies as having thick, striated walls and plugs at both ends (Fig. 65.16G).

Anisakis, Pseudoterranova, and *Contracaecum* spp. (Anisakiasis)

Ingestion of raw fish, although considered by many to be a delicacy, has resulted in an increase in the number of reported cases of fish nematode infections. *Anisakis* spp., *Pseudoterranova* spp., and *Contracaecum* spp. are common gastrointestinal parasites of marine mammals and fish-eating birds. Small, shrimplike crustaceans (krill) serve as the first intermediate host and the infective stages (L3 larvae) are found in many saltwater fish and squid paratenic hosts. With little or no host specificity for fish and molluscan hosts, most commercial marine fish and squid are possible sources for human infection. When ingested by humans, these L3 larvae may attempt to penetrate the wall of the stomach or small bowel, causing acute abdominal pain. Treatment is with surgical or endoscopic removal (Drugs for Parasitic Infections, 2013). Anisakiasis may be presumptively diagnosed based on an appropriate history and clinical findings. The condition may be confirmed by the recovery of an intact worm at endoscopy or by the presence of an eosinophilic granuloma containing an identifiable, usually dead and degrading, nematode in a biopsy specimen. It is believed that species of *Anisakis* appear to be more prone to produce invasive disease and end up in ectopic locations, whereas *Pseudoterranova* spp. tend to be coughed up or vomited intact and often alive. The species-level identification of larval anisakids is not possible (Mathison & da Silva, 2018). Serologic diagnosis may be achieved by a positive skin prick test in conjunction with clinical manifestations and a recent history of consumption of seafood. Positive skin-prick tests should be confirmed by specific IgE assays and a negative reaction to host fish proteins (Audicana & Kennedy, 2008).

CESTODES

Several species of cestode infect humans in their larval stages and may produce serious disease. The more commonly encountered ones are readily distinguishable from each other and have unique patterns of transmission. When seen in tissue sections, larval and adult stages of cestodes contain basophilic-staining laminated bodies known as *calcareous corpuscles*, which are an important aid in their recognition.

Taenia solium (Cysticercosis)

Human infection with the larval stage of the pork tapeworm, *T. solium*, is found worldwide and occurs following unintentional ingestion of the eggs in food and fomites contaminated with human feces. The disease is especially prevalent in regions of Mexico, South and Central America, Africa, India, and Asia that have poor sanitation and where free-roaming pigs have access to human feces containing *T. solium* eggs (CDC, 2018b). Most cases in the United States originate from highly endemic areas; however, locally acquired cases may occur when individuals with intestinal *T. solium* infection (taeniasis) contaminate food with eggs that are shed in their stool). Cysticercosis is one of the 5 NPIs targeted for public health action in the United States (CDC, 2018b).

Eggs that are accidentally ingested in contaminated food or water subsequently hatch in the gastrointestinal tract, and the released oncosphere penetrates the intestinal mucosa and disseminates via the bloodstream to distant sites, including the skeletal muscle, heart, brain, or eye, where symptoms of infection and inflammation may become especially apparent. Seizures are a common complication of neurocysticercosis in endemic areas and are often the presenting symptom (CDC, 2018b; White, 2018). Treatment is complex and depends on the location of the lesion(s) and extent of disease. Guidelines were recently published for diagnosing and treating neurocysticercosis (White, 2018).

The diagnosis is usually made on clinical grounds in endemic areas but may be much more difficult to establish in nonendemic settings. Radiographs are helpful in recognizing the presence of calcified cysts but not in recognizing recent infection. Both brain noncontrast computed tomography (CT) and magnetic resonance imaging (MRI) are recommended for evaluating and classifying the form of neurocysticercosis (White et al., 2018). Laboratory confirmation is best achieved by antibody detection by enzyme-linked immunotransfer blot due to its superior sensitivity over routinely available ELISAs using crude antigens (White et al., 2018).

Recovery of an intact cysticercus at the time of surgery also confirms the diagnosis. However, in cases of neurocysticercosis, invasive procedures are not desirable. The cysticercus, or "bladder worm," is a translucent, fluid-filled, oval sac containing a single inverted protoscolex that measures 5 mm or more in diameter (Figs. 65.22A and 65.22B).

The occurrence of cysticercosis in someone from a nonendemic area and without an appropriate travel history should be investigated for accidental exposure to individuals involved in food preparation or for the possibility of infection with a different *Taenia* species (Schantz et al., 1992; CDC, 2018b).

Echinococcus spp. (Hydatidosis)

Human infection with larval stages of tapeworms of the genus *Echinococcus* may take one of three forms: (1) unilocular hydatid disease caused by *Echinococcus granulosus*, *E. canadensis*, and *E. ortleppi*; (2) multilocular or alveolar hydatid disease caused by *E. multilocularis*; or (3) polycystic hydatid disease caused by *E. vogeli* and *E. oligarthra* (Romig et al., 2015). Dogs and other canids are definitive hosts for these minute tapeworms (although *E. oligarthra* uses wild felids as its natural definitive hosts). What they lack in size they make up for in numbers, with many hundreds or thousands of worms producing large numbers of eggs in one host. Eggs are passed in the stools and are ingested by the intermediate hosts, which include sheep, cattle, pigs, rodents, and other herbivorous animals. Humans are infected following accidental ingestion of eggs from the environment.

Eggs hatch in the intestine; the released oncospheres penetrate the intestinal wall and then enter the bloodstream. Although most hydatids develop in the liver, some disseminate to other sites. Development of the cysts is slow—it may take many years for a cyst measuring 10 to 15 cm in diameter to form. In the usual secondary hosts, the cysts contain numerous protoscoleces, which proliferate from a germinal membrane.

Members of the *E. granulosus*-complex, the most important species producing human disease, are common in many sheep- and cattle-raising areas of the world, including small regions of the United States, where dogs are the usual definitive host. Unilocular hydatids develop as single cysts in the liver and secondarily in the lungs or other locations. The cysts are filled with clear fluid and may contain smaller "daughter" cysts. Within the parent and daughter cysts are brood capsules and protoscoleces, which can number in the thousands (Fig. 65.24C). Symptoms in humans include a slowly growing mass lesion, although infection in space-limited locations such as bone and the CNS may become apparent earlier than in other sites. The preferred treatment modality is dependent on the size, location, and complexity of the lesion. The drug of choice is albendazole; it is usually administered in conjunction with aspiration or surgical removal of the cyst (Drugs for Parasitic Infections, 2013). The PAIR procedure (puncture, aspiration, injection and re-aspiration) with ultrasound guidance is now a preferred method of choice for single liver cysts (Drugs for Parasitic Infections, 2013).

The diagnosis is suggested on the basis of clinical presentation and history plus the use of radiography, CT scans, and ultrasonography. Serologic tests are very useful in confirming a diagnosis and usually involve a screening test such as EIA or IHA followed, if positive, by a confirmatory assay such as immunoblot or gel diffusion (Brunetti et al., 2010; Wilkins & Nutman, 2015). Sensitivity varies from 60% to 90%, depending on the characteristics of the case. False-positive reactions may occur with cysticercosis, although disease presentation should prevent confusion. Aspiration of cyst contents is potentially dangerous because spillage of cyst contents may result in dissemination of disease or, possibly, anaphylactic

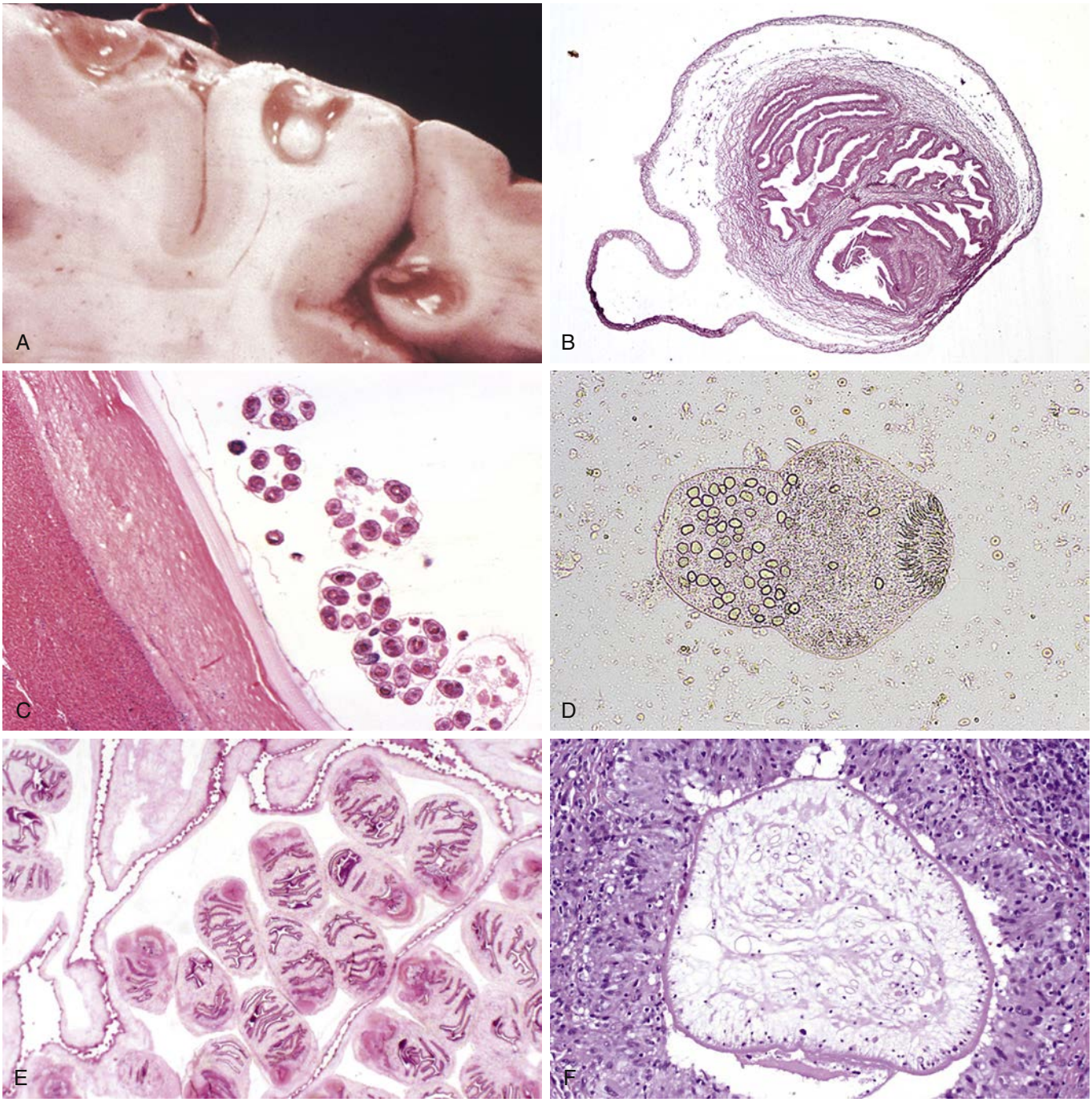

Figure 65.24 **A,** Gross specimen of neurocysticercosis. **B,** *Taenia solium* cysticercus (H&E; 10×). **C,** Hydatid cyst (*Echinococcus granulosus*) in the liver. Note the presence of multiple protoscoleces within brood capsules, a thin germinal membrane, a thicker laminated membrane, and the external fibrotic host reaction (10×). **D,** Protoscolex of *E. granulosus* found in aspirate fluid from a hepatic hydatid cyst. Note rostellar hooks and calcareous corpuscles (400×). **E,** Human infection with the larva of a canine *Taenia* (coenurus). Note the thin bladder membrane and numerous developing protoscoleces (10×). **F,** Sparganum (larva of *Spirometra* sp.) found in a lymph node. Note the surrounding granulomatous response (H&E; 100×). (**A** from Zaiman H: A pictorial presentation of parasites: a cooperative collection; https://www.astmh.org/education-resources/zaiman-slide-library. **C,** Courtesy of the Centers for Disease Control and Prevention Public Health Image Library.)

shock. However, if aspiration is performed, cyst contents usually reveal hydatid sand, a mixture of protoscoleces, disintegrating brood capsules, hooklets, and calcareous corpuscles (Fig. 65.24D). Aspiration performed under ultrasound or CT guidance seems to carry a lower risk and is now a commonly performed therapeutic method for patients with uncomplicated hydatid disease involving the liver.

Echinococcus multilocularis produces multilocular or alveolar hydatid disease in the northern regions of Europe and Russia, and in Alaska, Canada, and the northern tier of states in the United States. Intermediate hosts include several genera of small rodents; foxes, wolves, and dogs are definitive hosts. Human infection occurs in the liver, where the hydatid develops as an invasive cyst that insinuates itself within the tissue in an alveolar pattern without a surrounding outer capsule. Although the germinal membrane proliferates in the human liver, protoscoleces usually fail to develop. The pathologic picture is reminiscent of hepatic carcinoma. Given the more invasive nature of infection, surgical resection to the extent possible, in conjunction with prolonged albendazole administration, is the treatment of choice (Drugs for Parasitic Infections, 2013). Serologic assays that use *E. granulosus* antigens are useful in the diagnosis of this disease. The differential use of antigens from both parasites shows promise of discrimination between the two diseases (Brunetti et al., 2010; Wilkins & Nutman, 2015).

Echinococcus vogeli produces a polycystic hydatid cyst in humans that is invasive but, unlike *E. multilocularis*, produces both brood capsules and protoscoleces. The disease is limited to Latin America, where rodents, specifically the paca, and bush dogs complete the life cycle (D'Alessandro et al., 1979). Polycystic hydatid disease in South America may also be caused by *E. oligarthrus*, a parasite of felids and rodents. This species is similar morphologically to *E. vogeli* and cases have been misidentified (D'Alessandro et al., 1995). Treatment is not well defined for infection with these species.

Spirometra spp. (Sparganosis)

Sparganosis is caused by larval cestodes of the genus *Spirometra*, which are closely related to *Dibothriocephalus* spp. Adult stages commonly parasitize cats and dogs and their relatives in Asia (*Spirometra mansoni*) and North America (*Spirometra mansonoides*). Life cycles are similar to those of *Dibothriocephalus*: copepods serve as first intermediate hosts for procercoid larvae, and fish serve as second intermediate hosts for plerocercoid larvae. Humans become infected with the plerocercoid larval stage (also known as a sparganum) through ingestion of copepods in drinking water or ingestion of raw or incompletely cooked fish. Use of frogs and snakes as poultices may also result in the transfer of larvae to the human host. Sparganosis usually presents as localized or migratory subcutaneous swellings associated with erythema and pain, although brain infection may occur. Surgical exploration may reveal a delicate, slender, ivory-colored worm varying from a few to many centimeters in length. Cross-sections demonstrate a thick tegument with deep folds and parenchyma with prominent muscle bundles. No protoscolex is seen and calcareous corpuscles are numerous (Fig. 65.24F) (Orihel & Ash, 1995; Meyers, 2000; Pritt, 2018). Surgical removal is curative.

Taenia multiceps and *Taenia serialis* (Coenurosis)

Intestinal *Taenia* spp. of cats and dogs (primarily *Taenia multiceps* and *Taenia serialis*) produce a larval stage in intermediate hosts known as a *coenurus*. This stage consists of a large (up to 10 cm) transparent sac containing numerous scoleces that bud off from a germinal membrane and invaginate into the fluid-filled cyst (Fig. 65.24E). Sheep are the usual intermediate hosts for *T. multiceps*, and rodents, hares, and rabbits for *T. serialis*, although humans serve in this role through accidental ingestion of eggs originating from domestic cats and dogs. Similar to cysticerci, coenuri may develop in any organ, producing a similar disease. Most commonly, *T. multiceps* has a predilection for the CNS, while *T. serialis* is a predilection for subcutaneous tissues. Diagnosis is usually made by examination of the excised cyst or its demonstration in tissue sections. The presence of multiple invaginated scoleces within a single bladder differentiates the coenurus from the cysticercus of *T. solium*, which contains a single protoscolex. Surgical removal is curative.

TREMATODES

All liver-, lung-, and blood-inhabiting trematodes that mature in humans produce eggs that usually exit the body via stool, urine, or sputum. Because of their extraintestinal location, these flukes and their eggs may be found in tissues incidentally or in association with symptoms.

Adult *F. hepatica*, *C. sinensis*, and *O. viverrini* may be found in hepatic and biliary tissues, and occasionally in ectopic locations. The presence of typical eggs free in the tissues or within the uterus of the helminth often provides definitive identification. Adult *Paragonimus* spp. primarily reside in the lung but may be found in ectopic sites such as brain and subcutaneous tissue, where they produce abscesses, sometimes with large numbers of eggs. Adult schistosomes reside in blood vessels, primarily in the distribution of the inferior mesenteric vein (*S. mansoni*), the superior mesenteric vein (*S. japonicum* and *S. mekongi*), and the vesical plexus (*S. haematobium*). Although the adult stages are rarely encountered in tissue sections, eggs may be found in large numbers in tissues of the intestine, liver, and bladder (see Figs. 65.21B, 65.21D, and 65.21F). Eggs may disseminate via the bloodstream to other sites, including the brain, spinal cord, lungs, heart, kidneys, and spleen. The eggs of *S. japonicum* are especially prone to disseminate because of their smaller size and the large numbers typically produced. Identification of eggs is dependent on recognition of their typical sizes and morphologic characteristics (e.g., presence of a miracidium) in appropriate tissues.

MEDICALLY IMPORTANT ARTHROPODS

Arthropods comprise a large and diverse group of organisms, few of which have clinical or economic significance. Those that do, however, are important causes of morbidity and mortality in humans and their domestic animals and are responsible for serious economic losses to agriculture. Although perhaps best known among clinicians for their ability to transmit various infectious agents—including viruses, bacteria (rickettsia, spirochetes, and others), protozoa, and certain helminths—arthropods also cause serious disease by direct tissue invasion, envenomation, vesication, blood loss, and allergic reaction. Exaggerated fears of arthropods (entomophobia) and delusions of infestation (delusory parasitosis) are not uncommon neuroses, which may be disabling to some individuals. Species directly or indirectly responsible for human disease include representatives of all the major arthropod classes (Table 65.11).

In this section, an approach that the clinical laboratory may use when evaluating clinical specimens containing arthropods is presented, followed by a brief discussion of each of the arthropod groups of medical importance. A variety of general and specialized texts and guides are available for more complete coverage of the field of medical entomology (National Communicable Disease Center, 1969; Beaver et al., 1984; Lane et al., 1993; Moraru & Goddard, 2019; Mullen & Durden, 2018; Telford & Mathison, 2019), while a recent review (Mathison & Pritt, 2014) and bench guide (Mathison & Pritt, 2015) specifically discuss the identification of arthropods in the clinical microbiology laboratory.

BIOLOGICAL CHARACTERISTICS

Arthropods are characterized by a bilaterally symmetric, segmented body; several pairs of jointed appendages; and a rigid chitinous exoskeleton that is molted repeatedly during growth. Development proceeds from egg to adult through incomplete (hemimetabolous, with egg, nymph, and adult stages) or complete (holometabolous, with egg, larva, pupa, and adult stages) metamorphosis. Bedbugs, kissing bugs, lice, and cockroaches are examples of insects that undergo incomplete metamorphosis. Flies and mosquitoes, fleas, ants, bees, wasps, and beetles undergo complete metamorphosis; wormlike larval forms pupate to emerge as adults. Arachnids undergo developmental changes most similar to the process of gradual metamorphosis. The larval stages of those arthropods that undergo complete metamorphosis often prove to be the most difficult for clinical laboratorians to identify and should be referred to a medical entomologist if full identification is required for clinical management.

MECHANISMS OF INJURY

Direct Tissue Invasion

Invasion of superficial tissues (referred to as *infestation*) may occur with a variety of arthropods, of which scabies mites, chigoe fleas, and some dipteran larvae (maggots) are most common. Invasion of deeper body tissues and cavities (referred to as *infection*) occurs primarily with maggots and rarely with pentastomid larvae. Tissue invasion by dipteran larvae is referred to as *myiasis* and may occur in living or devitalized tissues depending on the involved species.

Envenomation

Many arthropods are capable of injecting venom with their bites or stings. For most individuals, these compounds cause only local tissue reactions, but serious, life-threatening reactions such as anaphylaxis may occur, often as a result of previous sensitization to the particular toxin. Hymenopteran (ants, bees, and wasps) and scorpion stings are among the greatest offenders (Moraru & Goddard, 2019). The bites of certain arthropods, especially centipedes; mosquitoes, flies, and biting midges; bedbugs, kissing bugs, and assassin bugs; sucking lice; fleas; and ticks and mites may also be toxic, causing local or systemic reactions. Almost all spiders are venomous, but only a few groups (widow spiders, violin spiders, and certain tarantulas, to name a few) pose significant health risks to humans. Less common but recognized causes of envenomation result from exposure to the urticating hairs of certain caterpillars and beetle larvae.

Vesication

Certain larger tropical millipedes are capable of spraying a vesicating (blister-causing) chemical substance from glands located on each body segment. These compounds are especially irritating should they reach the conjunctiva. Blister beetles are so named because of their ability to discharge vesicating fluids (cantharidin, the active ingredient in the aphrodisiac Spanish fly) from their bodies when handled.

Blood Loss

Arthropods responsible for producing significant irritation or blood loss to humans and domestic animals include bedbugs, kissing bugs, lice, fleas, flies, mosquitoes, biting midges, ticks, and mites. Although these activities are rarely life-threatening, the concurrent transmission of infectious agents may be.

Transmission of Infectious Agents

Many arthropods play an integral role in the mechanical or biological transmission of infectious disease agents. The common housefly, *Musca domestica*, may be responsible for the mechanical transmission of agents of bacillary dysentery, cholera, typhoid, viral diarrhea, amebic dysentery, and giardiasis, as well as pinworms and tapeworms. Mechanisms involved in the biological transmission of infectious agents vary from simple organism amplification in the arthropod vector to more complex life cycle changes in the involved parasite. Ticks and mites are involved in the transmission of certain bacteria (*Borrelia*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, and others), protozoa (*Babesia*), and viruses. Among insects, lice are involved in the transmission of bacteria (*Rickettsia*, *Bartonella*, and *Borrelia*); kissing bugs

TABLE 65.11

Overview of the Medical Importance of Major Groups of Arthropods

Major Arthropod Groups	Medical Importance
Crustacea (crustaceans)	
Copepoda (copepods)	Intermediate host of *Spirometra*, *Gnathostoma*, *Dracunculus medinensis*
Decapoda (crabs, crayfish)	Intermediate hosts of *Paragonimus*
Pentastomida (tongueworms)	Causal agents of visceral pentastomiasis
Chilopoda (centipedes)	Envenomation
Diplopoda (millipedes)	Expulsion of noxious chemicals; intermediate hosts for acanthocephalans
Hexapoda (insects)	
Psocodea (lice)	
Pediculus (head and body lice)	Nuisance pests; vectors of agents of epidemic typhus, trench fever, louse-borne relapsing fever
Pthirus (pubic lice)	Nuisance pests
Blattodea (cockroaches)	Nuisance pests; intermediate hosts of acanthocephalans
Hemiptera (true bugs)	
Cimex (bed and bat bugs)	Nuisance pests
Triatoma, *Panstrongylus*, *Rhodnius* (kissing bugs)	Vectors of agent of Chagas disease
Siphonaptera (fleas)	
Xenopsylla (oriental rat flea)	Vector of agent of plague, murine (endemic) typhus
Ctenocephalides (cat and dog fleas)	Vectors of feline rickettsiae; intermediate host for *Dipylidium*, *Hymenolepis*
Pulex (human flea)	Nuisance pest; intermediate host of *Dipylidium*, *Hymenolepis*
Tunga (chigoe fleas)	Agents of tungiasis
Diptera (flies)	
Aedes, *Culex*, *Anopheles*, *Mansonia* (mosquitoes)	Vectors of agents of lymphatic filariasis, dirofilariasis, malaria, dengue, yellow fever, West Nile, chikungunya, SLE, EEE, WEE, many other arboviruses
Culicoides (biting midges)	Vectors of agents of mansonellosis
Phlebotemus, *Lutzomyia* (sand flies)	Vectors of agents of leishmaniasis, bartonellosis, phlebovirus
Simulium (black flies)	Vectors of agents of onchocerciasis, mansonellosis, dirofilariasis
Glossina (tse tse flies)	Vectors of agents of African sleeping sickness
Chrysops (deer flies)	Vectors of agents of loiasis, tularemia
Musca, *Fannia* (house, little house flies)	Vectors of agents of salmonellosis, shigellosis, *E. coli*–associated illness, cholera, bacterial conjunctivitis, thelaziasis; agents of myiasis
Dermatobia, *Oestrus*, *Cuterebra* (bot flies); *Sarcophaga*, *Wohlfahrtia* (flesh flies); *Cordylobia*, *Cochliomyia*, *Chrysomya*, *Lucilia*, *Phormia* (blow flies); others	Agents of myiasis
Hymenoptera (bees, ants, wasps)	Envenomation, allergic reactions to stings
Lepidoptera (butterflies and moths)	Caterpillars with urticating hairs
Coleoptera (beetles)	Expulsion of noxious chemicals; intermediate hosts for *Hymenolepis*, acanthocephalans
Arachnida	
Acari (ticks and mites)	
Amblyomma, *Ixodes*, *Hyalomma*, *Dermacentor*, *Rhipicephalus* (hard ticks)	Vectors of agents of Lyme disease, babesiosis, human granulocytic ehrlichiosis, Boutonneuse fever, human monocytic ehrlichiosis, Rocky Mountain spotted fever, tularemia, Crimean-Congo hemorrhagic fever, Colorado tick fever, relapsing fever borreliosis, human granulocytic anaplasmosis, tidewater spotted fever, Powassan virus, several others; implicated in tick paralysis and alpha-Gal syndrome
Ornithodoros, *Carios*, *Otobius* (soft ticks)	Vectors of agents of tick-borne relapsing fever
Sarcoptes scabiei	Agent of scabies
Demodex spp. (follicle mites)	Commensals on the human host
Various avian and rodent mites	Nuisance pests; vectors of agent of rickettsialpox
Chiggers	Nuisance pests; vectors of agent of scrub typhus
Scorpions	Envenomation
Spiders	Envenomation

Modified from Mathison BA, Pritt BS: Laboratory identification of arthropod ectoparasites, *Clin Microbiol Rev* 27:48–67, 2014; and Telford III SR, Mathison BA: Arthropods of medical importance. In Carroll JC, Pfaller MA, Landry ML, et al., editors: *Manual of clinical microbiology*, ed 12, Washington, D.C., 2019, ASM Press.

transmit trypanosomes; fleas transmit the agents of plague, typhus, and tapeworm infections; and dipterans transmit arboviruses, bacteria, and parasitologic agents of malaria, African trypanosomiasis, leishmaniasis, and filariases (see also Table 65.11).

Hypersensitivity Reactions

Most serious reactions to arthropod bites and stings result from allergic hypersensitivities. Hymenopteran stings alone are responsible for most arthropod-related deaths and usually result from the development of hypersensitivity following repeated exposure to venom. Allergies may be exacerbated following exposure to the saliva, excrement, or body parts of mites, ticks, lice, bedbugs, caterpillars, moths, and butterflies. Asthma and hay fever may also develop in response to the presence of the large variety of house, dust, and animal mites in the environment (Mullen & Durden, 2018).

Psychological Manifestations

Entomophobia refers to an unreasonable or excessive fear of seeing or touching arthropods. Although this fear may occasionally result in disruption of a

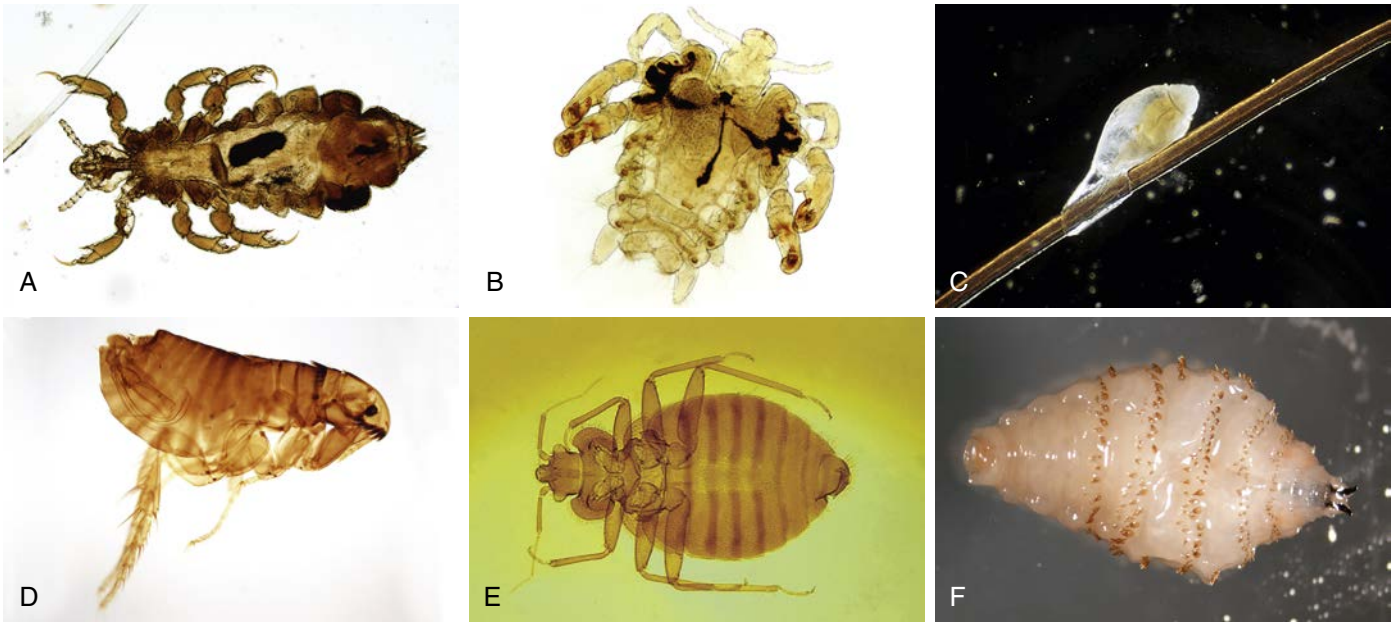

Figure 65.25 Medically important insects. **A,** *Pediculus humanus capitis*, the head louse, holding a strand of hair with one leg. **B,** *Pthirus pubis*, the crab louse. **C,** Intact egg (nit) of *P. pubis*. **D,** *Ctenocephalides* sp., the cat and dog fleas. Note the powerful hind legs. **E,** *Cimex lectularius*, the common bedbug. **F,** Larva of *Dermatobia hominis*, the "human" botfly. Note the two sclerotized hooks at the anterior end and numerous body spines (10×). (**D** *and* **F**, *with permission from the College of American Pathologists Arthropod Bench Reference Guide;* **E,** *with permission from Murray PR, Barron EJ, Pfaller MA, et al., editors:* Manual of clinical microbiology, *ed 6, Washington, DC, 1995, ASM Press.*)

person's normal activities, it rarely becomes incapacitating. Delusory parasitosis is a more serious emotional disorder in which an individual is convinced of being infected with parasites or arthropods despite objective evidence to the contrary. As the delusion progresses, the individual may report loss of employment, divorce, repeated use of pesticide services, and movement from house to house. Visits to health care providers are usually numerous and unsatisfactory. The problem may originate in the home or workplace and may be transferable from one to the other. The delusion may be so convincing that other family members or friends may believe it or acquire it themselves. The patient may submit to the laboratory numerous specimens, such as skin, fabric, lint, hair, and mucus. It is incumbent on laboratory personnel to examine these materials to rule out true infestation. The mysterious onset of irritation and itching may be due to bites from unrecognized scabies mites, lice, fleas, or bedbugs, or from insects and mites questing from an abandoned rodent or bird nest in an area of human habitation. Before such causes are dismissed, they must be looked for and their presence excluded. Patients with delusional parasitosis are difficult to treat, although the use of antipsychotics may provide some relief. The lack of clarity as to symptomatology, etiology, and diagnosis creates difficulty in management for both the patient and health care provider (Campbell et al., 2019).

LABORATORY APPROACHES TO ARTHROPOD IDENTIFICATION

Arthropod specimens are often directed to the clinical laboratory by both clinicians and patients with the expectation that they can be accurately identified, but few laboratory personnel receive more than a cursory exposure to entomology during training. Nonetheless, laboratorians should have access to texts and dichotomous keys, which should allow limited identification of the more commonly encountered medically important groups, especially ectoparasites (fleas, lice, mites, and ticks). Of greater importance is the ability of laboratory personnel to recognize those rare situations in which outside expertise should be sought. This specifically relates to those occasions when significant clinical decisions regarding therapy and prognosis are being made. State or local public health laboratories often have the expertise available or know of individuals trained in medical entomology who can be reached at regional educational institutions, museums, or other public or private agencies, including the CDC.

Specimens submitted to the laboratory are most often intact organisms, skin scrapings, tissues, sputum, urine, or stool. Inanimate objects, including foodstuffs, water, clothing, bedding, and carpeting, among others, may also be submitted. It is not uncommon for patients to submit arthropods recovered from the toilet bowl following urination or a bowel movement. In most cases, the presence of such organisms is incidental and is not related to infection.

Proper killing and preservation of arthropods is important to preserve those characteristics necessary for identification. Small, nonwinged arthropods—especially ectoparasites (lice, fleas, ticks, and mites), larval forms (maggots, grubs, and caterpillars), spiders, and scorpions—should be placed directly into 70% to 80% ethanol. Large larval forms are best killed in hot (not boiling) water to extend their bodies and prevent contraction before immersion in alcohol. Attached tissue or other debris should be gently removed or washed away prior to preservation. Smaller forms (mites, small ticks, fleas, and sandflies) may be prepared as permanent slide mounts (Mathison & Pritt 2014, 2015).

Winged insects—especially adult mosquitoes, midges, and flies—should be killed by exposure to the fumes of ethyl acetate or chloroform and preserved dry to retain the taxonomic information contained in the body and wing scales. Additional details regarding the collection, preservation, and preparation of arthropod specimens for examination are found elsewhere (Johnson & Triplehorn, 2005).

INSECTS

Insects account for more than 90% of all described arthropod species, although few are responsible for human disease. Members of this class are distinguished from other arthropods by having a body divided into three parts (head, thorax, abdomen); one pair of antennae; three pairs of legs; and one, two, or no pairs of wings. This is the only group of arthropods that has developed the ability of flight.

Parasitic Lice

Parasitic lice are dorsoventrally flattened, wingless insects that have characteristic claws on the ends of each leg that allow attachment to body hairs or clothing (Figs. 65.25A and 65.25B). All species suck blood intermittently, which may cause unexplained dermatitis. Eggs, known as *nits*, are deposited on hair shafts or clothing depending on the species. Although named for their primary site of attachment, they do not always remain confined to that location. The head louse, *Pediculus humanus capitis*, and the body louse, *Pediculus humanus humanus*, are indistinguishable to the nonspecialist and are thought to actually represent two ecotypes of the same species based on newly acquired molecular data. They are longer than they are wide and grow to about 3 mm in length. Biological differences are apparent; only *P. h. humanus* transmits the agents of epidemic typhus, trench fever, and relapsing fever (Moraru & Goddard, 2019). Infestations with *P. h. humanus* occurs primarily in settings of war, poverty, and homelessness, where bathing and laundering do not regularly occur. In comparison, *P. h. capitis* is much more common, particularly among children of school age. Children are at particular risk for acquiring head lice through the sharing of caps, clothing, and combs (Mullen & Durden, 2018). Nits of head lice are deposited primarily on hair shafts; those of body lice are deposited on clothing or other fomites. Because objects such as hair casts, dander, hair spray, and fungal hair infection may mimic nits, differentiation is important. Nits

are typically 1 mm long and when unhatched have intact opercula that are raised and flat anteriorly. Transmission occurs primarily through the sharing of infested clothing and bedding because body lice tend to lay their eggs in clusters, especially along seams or waistbands. The pubic louse, *Pthirus pubis*, is distinctly different from the others; it is wider (measuring up to 2 mm in width), the abdomen is more crablike, and the first pair of legs is significantly smaller and more slender than the other pairs (see Fig. 65.25B). Pubic lice and their nits, which are raised and conical (Fig. 65.25C) are found primarily on pubic hairs but may extend to other coarse hairs, including those on the chest, armpit, and facial hair. Transmission occurs primarily during sexual intercourse or other direct person-to-person contact. Lice infestation is treated with topical pediculicides.

Fleas

Fleas are small (1–2 mm), laterally compressed, wingless ectoparasites capable of sucking blood (Fig. 65.25D). Long, muscular legs are adapted for jumping great distances. Fleas that attack humans are parasites of other mammals or poultry and include both blood-sucking pests (many species) and tissue-penetrating jiggers. Infestations commonly occur with exposure to domestic animals and pets; the most pestiferous species are the dog flea (*Ctenocephalides canis*), the cat flea (*C. felis*), and the human flea (*Pulex irritans*). Some individuals become highly sensitized to flea bites, whereas others are unaffected. Cat and dog fleas are the usual intermediate hosts for the tapeworm *D. caninum* and less frequently for *H. diminuta* and *H. nana*. Because larvae of these species often develop in an animal's bedding or in carpets and furniture, eradication may require fumigation and cleaning of those articles. The Oriental rat flea, *Xenopsylla cheopis*, is an extremely important species because it transmits the agents of plague (*Yersinia pestis*) and murine typhus (*Rickettsia typhi*). Although normally parasitizing several species of rats, this flea readily attacks humans should the rodent host die. The jigger or chigoe fleas *Tunga penetrans* and *T. trimamillata* are found in both Central and South America, with *T. penetrans* having been introduced to regions of tropical Africa and Asia. The female flea attaches to and embeds itself in the skin, especially between the toes and under the toenails, where it grows to the size of a small pea. After eggs are discharged, the flea dies, prompting an inflammatory response and possible secondary bacterial infection or myiasis. Tungiasis is diagnosed by identifying the dark portion of the flea's abdomen (displaying the spiracles) protruding from the skin surface of an enlarging lesion (Moraru & Goddard, 2019) or by histopathologic examination of skin biopsies (Meyers et al., 2013; Pritt, 2018). Liberated eggs may be submitted to the clinical laboratory for identification (Mathison & Pritt, 2015).

Cockroaches

Cockroaches have closely adapted themselves to human habitation, sharing our food, shelter, and warmth. Although they are primarily nuisance pests, cockroaches are potential carriers of fecal pathogens owing to their ability to move quickly from sewers and drains to food preparation areas. In addition to transmitting pathogenic bacteria, they may spread hepatitis and poliovirus; intestinal protozoa, including *E. histolytica*; and several species of enteric nematodes. Allergies and asthma may develop in some individuals following exposure to the excreta, cast skins, or body parts of cockroaches (Moraru & Goddard, 2019).

Bed Bugs and Kissing Bugs

Bed bugs (family *Cimicidae*) and kissing bugs (family *Reduviidae*) are blood-sucking insects that have a long, narrow proboscis that is folded underneath the body when not in use. Bedbugs (*Cimex lectularius* and *C. hemipterus*) are reddish brown, dorsoventrally flattened, wingless insects approximately 5 mm in length (Fig. 65.25E). They are cosmopolitan in distribution and attack most any mammal, feeding primarily at night. During daylight hours, they hide under mattresses, loose wallpaper, and floorboards. Although they are not known to transmit disease-causing agents, bedbug bites may cause painful weals or bullae, depending on an individual's sensitivity to their saliva.

Kissing bugs (*Triatoma*, *Rhodnius*, *Panstrongylus*) have a cone-shaped head on a narrow neck and an abdomen that is widened in the middle. These insects are black or brown, and some have orange and black markings on the abdomen. They average 1 to 3 cm in length and, unlike bedbugs, have well-developed wings for flight. Similar to bedbugs, kissing bugs are relatively painless feeders on vertebrates and produce similar skin reactions. In Mexico and Central and South America, they transmit the agent of Chagas disease, *T. cruzi*, in the feces, which is secondarily inoculated into the skin by the human host while scratching (Moraru & Goddard, 2019). Although they are also found throughout the southern United States, autochthonous transmission of Chagas disease is thought to occur only rarely.

Bees, Wasps, and Ants

Hymenopterans are social insects that readily defend their nests when disturbed. In nonreproductive females, the ovipositor is modified as a stinger capable of injecting venom for use in the capture of prey or for defense. The venom of bees, wasps, hornets, and yellow jackets causes only transient swelling and discomfort in most individuals but may be responsible for systemic reactions, including anaphylaxis, in others who were previously sensitized (Moraru & Goddard, 2019). Up to 100 people in the United States die each year from hymenopteran stings. Many species of ant are problematic for humans because of their ability to sting. Some groups, such as harvester and fire ants, are capable of giving painful stings.

Beetles

Although beetles are perhaps best known as pests of agricultural crops, some species may give a painful bite. Others, especially blister beetles, may exude vesicating fluids (cantharidin) that cause dermatitis or blister formation. The larvae of certain larder beetles have urticating hairs that may be responsible for dermatitis or, if ingested, irritation of the gastrointestinal tract. Larval and adult larder and grain beetles also may serve as intermediate hosts for the rodent tapeworms *H. diminuta* and *H. nana*. Scarab beetles serve as intermediate hosts for the acanthocephalan, *Macracanthorhynchus hirudinaceus*.

Moths and Butterflies

Certain larvae (caterpillars) of *Lepidoptera* possess urticating hairs or spines capable of injecting venom when handled. Although most effects of these toxins remain localized to the skin, systemic effects such as shock and paralysis have been reported (Moraru & Goddard, 2019). Adult tussock and gypsy moths are known to have urticating scales and hairs that may cause dermatitis, eye irritation, or respiratory tract irritation, especially among forestry workers (Shama et al., 1982).

Flies, Mosquitoes, and Midges

Diptera are characterized by the presence of a single pair of membranous wings used for flight. Among all arthropods, they are responsible for the greatest share of human disease through blood-sucking activities, biological or mechanical transmission of infectious agents, and direct tissue invasion by larval forms (myiasis). Bites from a variety of flies, mosquitoes, and biting midges often cause local irritation from sensitivity to the saliva and, in some individuals, systemic reactions. In addition to blood-sucking activities, the repeated attacks themselves may be physically and psychologically damaging. Certain blood-sucking species are also responsible for the transmission of important human pathogens, including those that cause malaria, lymphatic filariasis, and arboviral disease by mosquitoes; onchocerciasis by black flies; loiasis by deer flies; leishmaniasis and bartonellosis by sandflies; and African trypanosomiasis by tsetse flies. Other viral, bacterial, and parasitic agents are readily transmitted mechanically by nonbiting flies such as house flies, flesh flies, and blow flies, which can easily contaminate human food.

Myiasis may occur in an accidental, facultative, or obligatory fashion. The housefly, *Musca domestica*, has no requirement for developing in mammalian tissue, yet may be found occasionally in dead tissue or under plaster casts. This type of accidental myiasis is not uncommon but rarely is clinically significant. Facultative myiasis is most often caused by blow flies and flesh flies, which ordinarily feed on dead tissues but may move into adjacent viable tissues. Obligatory myiasis is caused by certain species that develop only in living tissues. Those species that infect humans are all of zoonotic origin. The human bot fly, *Dermatobia hominis*, develops in boil-like subcutaneous lesions, with the posterior end of the maggot appearing at the skin surface (Fig. 65.25F). This species is most commonly found in individuals who have spent time in Central or South America. It is unusual in that its eggs are mechanically transported to the host by other flying insects, usually mosquitoes. The tumbu fly (*Cordylobia anthropophaga*), found in sub-Saharan Africa, causes a furuncular type of myiasis. Eggs of this species usually are laid on the ground or on hanging laundry, and larvae rapidly penetrate the skin on contact. The most serious myiasis is caused by the Old World screwworm, *Chrysomyia bezziana*, and the New World screwworm, *Cochliomyia hominivorax*. These species lay their eggs directly on their cattle hosts, usually on wounds or near the nostrils. The larvae actively feed on and move through living tissues. Human infection may be particularly destructive if larvae invade the eye, nose, or mouth. In North America, zoonotic furuncular or visceral myiasis caused by *Cuterebra* species (normally parasites of rodents and rabbits), is not rare. Other species may also be responsible for traumatic, obligatory myiasis in humans (Francesconi & Lupi, 2012).

ARACHNIDS

Medically important arachnids include scorpions, spiders, ticks, and mites. Scorpions and spiders have two body segments, the cephalothorax and the

Figure 65.26 Medically important arachnids. **A,** Scorpion. Note the forward-directed pincer claws and stinger on the tail tip. **B,** *Dermacentor variabilis* (dog tick), nonengorged adult female. **C,** *Ixodes scapularis* (black-legged tick or deer tick), nonengorged adult female. **D,** *Ornithodoros* sp. (soft tick), nonengorged adult, ventral view. **E,** *Sarcoptes scabiei* (itch mite) adult. Note the presence of adjacent eggs in skin scrapings. **F,** *Demodex folliculorum* (follicle mite) adult. (**A,** *With permission from Murray PR, Barron EJ, Pfaller MA, et al., editors:* Manual of clinical microbiology, *ed 6, Washington, DC, 1995, ASM Press.* **B** *and* **C,** *With permission from Northwest Infectious Disease Consultants.* **E,** *With permission from Spach DH, Fritsche TR: Norwegian scabies in a patient with AIDS,* N Engl J Med *331:777, 1994.*)

abdomen, whereas ticks and mites have only one. Members of the group have four pairs of legs as nymphs and adults; larval ticks and mites have three pairs of legs. All lack antennae, mandibles, and wings. Scorpions and spiders are best known for their ability to inject poisonous venom, whereas ticks and mites are best known as vectors for viral, bacterial, and protozoan pathogens.

Scorpions

Unlike other arachnids, scorpions have a pair of forward-directed pincer claws that impart a crablike appearance and a segmented tail with a bulbous stinging apparatus in the tip (Fig. 65.26A). They are predatory in nature and paralyze their intended victim with venom from the sting, which may also be used for defensive purposes. Toxicity to humans varies depending on the species; many elicit no more reaction than that of a bee sting, but some are deadly, causing more than 1000 deaths annually. Poisonous species are found in the Western Hemisphere, southern Europe, Africa, Australia, and the Middle East (Moraru & Goddard, 2019).

Spiders

Spiders lack a tail with an attached stinger but instead have fanglike chelicerae among their mouthparts, through which venom can be expressed. Although most spiders are venomous, few have chelicerae capable of penetrating human skin. Most spider bites cause only transitory irritation and pain. Widow spiders (genus *Lactrodectus*) are one group responsible for systemic arachnidism through the action of a potent neurotoxin capable of producing weakness, myalgia, paralysis, convulsions, and, occasionally, death. Published mortality rates vary from less than 1% to 6%. Five closely related species are found in the United States; the southern black widow (*Lactrodectus mactans*) is the most widespread. Female black widow spiders are glossy black with a characteristic red or orange hourglass-shaped marking on the underside of the abdomen and have a leg span of 3 to 4 cm. They live in protected locations such as woodsheds, basements, and outdoor privies (Moraru & Goddard, 2019).

Violin spiders (genus *Loxosceles*) are responsible for necrotic arachnidism or loxoscelism. In the United States, the brown recluse or fiddleback spider (*Loxosceles reclusa*) is most often involved, although other species are present. This species is 1 to 2 cm long and tan to dark brown; it has a darkened, violin-shaped marking oriented base forward on the dorsum of the cephalothorax. When present in homes, violin spiders are reclusive in their habits, preferring undisturbed areas such as closets, basements, and under porches. Their bite is painless and often goes unrecognized until several hours later, when the area becomes red, swollen, and painful. The venom is dermonecrotic and hemolytic, producing cutaneous necrosis and sloughing of involved skin over several days. The resulting lesion may be difficult to heal and subject to secondary infection. Systemic reactions such as hemolysis and acute renal failure are rare. Other spider genera have also been implicated in producing necrotic arachnidism (Moraru & Goddard, 2019).

Ticks

Ticks are one of the more common arthropods to be submitted to the clinical parasitology laboratory for identification. Unlike spiders and scorpions, ticks have a fused cephalothorax and abdomen, as well as a characteristic toothed hypostome for feeding. Tick development progresses through four stages: egg, larva, nymph, and adult (soft ticks may have multiple nymphal stages). Following hatching, a blood meal is required for progression to the subsequent stage. Humans usually acquire ticks in grassy or brushy areas in close proximity to the usual animal hosts. All species are obligate blood-sucking ectoparasites and are important vectors of viral, bacterial, and protozoan pathogens to humans and domestic animals. Their feeding activities may produce local tissue damage and blood loss, especially to livestock and wildlife, or tick paralysis, a syndrome caused by a neurotoxin secreted by a tick's salivary glands that produces ascending flaccid paralysis and toxemia. Symptoms may closely mimic those of Guillain-Barré syndrome, poliomyelitis, or botulism. Removal of the attached tick

usually results in resolution of symptoms within hours to days. Ticks have also been implicated in alpha-Gal syndrome, an allergic reaction to the carbohydrate Galα1-2Galβ1-(3)4GlcNAc-R (α-Gal) that is present in nonprimate mammals. When humans are bitten by a tick that has recently fed on a nonprimate mammal (e.g., deer), α-Gal can be injected into the bloodstream, resulting in antibody production to the carbohydrate. After this initial reaction, ingestion of animal meat with α-Gal causes an allergic reaction (de la Fuente et al., 2019; Wilson et al., 2019).

Species affecting humans include members of the family *Ixodidae* (hard ticks) and *Argasidae* (soft ticks). Hard ticks have anteriorly directed mouthparts and a sclerotized plate, or scutum, on the dorsum. The scutum covers the entire dorsum in the adult male but only the anterior portion in the adult female, larvae, and nymphs, allowing the body to swell when engorged (Figs. 65.26B and 65.26C). Argasid ticks have a soft leathery body lacking a scutum and ventrally directed mouthparts that are not visible when viewed from above (Fig. 65.26D). Unengorged ticks are generally 2 to 5 mm long but may enlarge to several times that size following engorgement.

Most ticks found crawling on or embedded in human skin are hard ticks. Soft ticks tend to feed only briefly and then often at night. Important species of hard ticks in North America include *Dermacentor variabilis* (American dog tick), *D. andersoni* (Rocky Mountain wood tick), *Amblyomma americanum* (Lone Star tick), *A. maculatum* (Gulf Coast tick), *Rhipicephalus sanguineus* (brown dog tick), *Ixodes scapularis* and *I. dammini* (black-legged or deer ticks), and *I. pacificus* (Western black-legged or deer tick). *Dermacentor* and *Amblyomma* ticks are called *ornate* ticks because of the presence of white markings on their scuta as adults; the other species are inornate ticks.

Dermacentor ticks transmit the agents of Rocky Mountain spotted fever, tularemia, Colorado tick fever, and, possibly, Q fever. *Ixodes* ticks are vectors of agents of Lyme disease, babesiosis, anaplasmosis, and Powassan virus. In other parts of the world, these ticks are responsible for the transmission of certain arboviruses. *Amblyomma* ticks are capable of transmitting agents of Rocky Mountain spotted fever, tidewater spotted fever, ehrlichiosis, and tularemia, and have been implicated in alpha-Gal syndrome. All these genera are capable of causing tick paralysis. *Rhipicephalus* ticks have been implicated in the transmission of agents of Rocky Mountain spotted fever and ehrlichiosis in North America, and of boutonneuse fever in the Mediterranean area. *Hyalomma* species transmit agents of Crimean Congo hemorrhagic fever in the Old World. Soft ticks of the genus *Ornithodoros* occur in many parts of the world, including the United States, and are important vectors of the relapsing fever spirochetes (*Borrelia recurrentis* and related species) (Moraru & Goddard, 2019; Mathison & Pritt, 2014; Muller & Durden, 2018).

Mites

Mites are arachnids of microscopic size (usually <1 mm) that are widely distributed in the environment. Medically important species may attack humans directly, serve as vectors for infectious disease, or cause dust allergies. Humans are commonly infested with both *Demodex folliculorum* and *D. brevis*, the follicle mites, and *Sarcoptes scabiei*, the itch or mange mite. Follicle mites are minute (0.1–0.4 mm), elongate parasites with stubby legs that can be recovered from hair follicles and sebaceous glands (Fig. 65.26F). They are common incidental findings on histologic skin preparations. Although their presence has been associated with various skin conditions, they are commonly found in healthy individuals as well, which makes their significance difficult to assess (Moraru & Goddard, 2019).

Sarcoptes scabiei mites are of greater medical importance because of their ability to create serpiginous tunnels through the upper layers of the epidermis. Transmitted through personal contact, these mites are found primarily in the interdigital spaces and the flexor surfaces of the wrists and forearms and less commonly in other areas, including the breasts, buttocks, and external genitalia. Inflammation and intense itching result from the tunneling activity and from the deposition of eggs and excreta. Clinical manifestations vary depending on the degree of sensitization to the parasites and their products. Lesions often become secondarily infected. Generalized dermatitis occurring in the presence of thousands of mites, typically in older adults or immunocompromised individuals, is known as *crusted* or *Norwegian* scabies. Classical scabies is commonly treated with topical 5% permethrin or 10% crotamiton, whereas oral ivermectin is added for cases of crusted scabies (Drugs for Parasitic Infections, 2013).

The diagnosis is made by placing skin scrapings collected from tunneled areas in 20% potassium hydroxide or mineral oil for clearing and examining under the microscope. Detection of eggs, six-legged larvae, and eight-legged nymphs or adults is diagnostic but may be difficult to demonstrate (Figs. 65.26E and 65.27). The diagnosis of scabies in an institutional or school setting may result in pseudoepidemics, in which numerous individuals develop itching without evidence of disease. Care must be exercised to properly diagnose the disease to identify real cases and differentiate them from cases of delusory parasitosis (Moraru & Goddard, 2019).

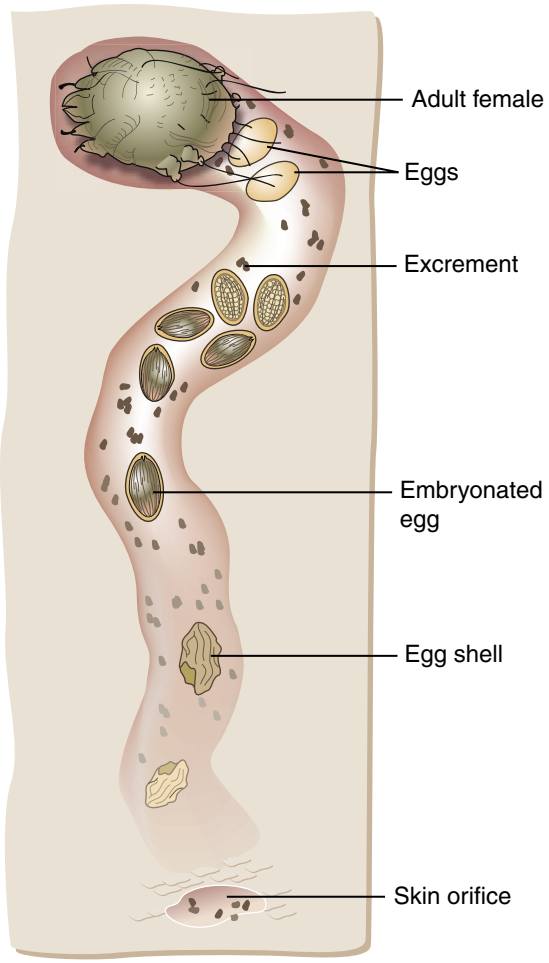

Figure 65.27 *Sarcoptes scabiei*. Diagram of a subcutaneous burrow.

A number of animal mite species may attack humans for a blood meal, either as larval forms or as adults, when normal mammalian or bird hosts are not available. Larval chigger mites (family *Trombiculidae*) are problematic in many parts of the world because their saliva can produce large wheal-and-flare reactions with intense itching. These tiny, six-legged larvae are often red and commonly attach to the skin in areas where clothing is restrictive, such as at the ankles, waistline, armpits, and wrists. In parts of Asia and Australia, trombiculid mites are vectors for the transmission of the agent of scrub typhus (Moraru & Goddard, 2019; Mullen & Durden, 2018). Avian and rodent mites in the genera *Ornithonyssus*, *Laelops*, *Dermanyssus*, and *Liponyssoides* (among others) will occasionally bite humans in the absence of, or during infestations of, their natural hosts. These mites cannot survive on human blood and are not vectors of disease-causing agents. One notable exception is the house mouse mite, *Liponyssoides sanguineus*, which has been implicated in the transmission of *Rickettsia akari* (rickettsialpox) in northeastern North America.

Certain nonbiting mites play a role in allergic rhinitis, asthma, and some skin conditions. The secretions, excreta, and body parts of the dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*, and the house itch mite, *Glycyphagus domesticus*, are potent allergens that may occur in great numbers in the household environment (Calderón et al., 2015). Routine testing by an allergist may identify the offending agent.

Mites can have a very similar appearance to ticks, particularly in their larval form, but can be differentiated by their lack of a toothed hypostome (attachment organ used by ticks) and lack of Haller's organ on their forelegs.

CLASSES OF LESSER MEDICAL IMPORTANCE

Millipedes

Millipedes are cylindrical arthropods with numerous apparent body segments, each with two pairs of legs; they are commonly found in and around decaying vegetation. Although they lack mouthparts capable of producing serious bites, many species produce vesicating secretions from glands located on each body segment. When handled roughly, the larger tropical

TABLE 65.12
Parasitic Infections in Compromised Hosts

Infection	Predisposing Host Abnormalities	Comments	References
Intestinal Protozoa			
Cryptosporidiosis	AIDS (especially CD4⁺ <200/μL), transplantation, antineoplastic chemotherapy	Severe protracted diarrhea (up to 17 L/day). May have extraintestinal involvement, including pancreas, biliary tract, and lungs. Limited therapy available; is not curative.	Wang et al., 2018; Xiao & Cama, 2019
Cystoisosporiasis	AIDS, transplantation, antineoplastic chemotherapy	Severe protracted diarrhea. Extraintestinal involvement of regional lymph nodes may be seen. Effective therapy is available.	Lindsay & Weiss, 2019
Cyclosporiasis	AIDS, probably other immunosuppression	Severe protracted diarrhea clinically similar to cryptosporidiosis and isosporiasis. Responds to trimethoprim-sulfamethoxazole.	Ortega & Sanchez, 2010; Lindsay & Weiss, 2019
Giardiasis	Common variable immunodeficiency, X-linked agammaglobulinemia	Prolonged diarrhea with malabsorption. AIDS is not a predisposing factor.	Saurabh et al., 2017
Blood and Tissue Protozoa			
Granulomatous amebic encephalitis	AIDS and other immunocompromised states	Usually caused by amebae of the genera *Acanthamoeba* or *Balamuthia.* Produces subacute or chronic central nervous system infection but may be acute in severely immunosuppressed hosts with dissemination	Visvesvara et al., 2007; La Hoz et al., 2019
Toxoplasmosis	AIDS with CD4⁺ usually <100/μL and other immunocompromised states; heart transplant, donor seropositive and recipient seronegative	Usually result of reactivation of cysts from previous infection. Often disseminated disease with multiorgan involvement or multifocal central nervous system lesions. Can cause pneumonitis resembling that caused by *Pneumocystis jiroveci*. May cause chorioretinitis. Can occur after transplantation of bone marrow but usually mild. Heart transplant with donor serologically positive and recipient serologically negative may lead to severe, often fatal toxoplasmosis.	Dubey & Jones, 2008; La Hoz et al., 2019
Leishmaniasis	AIDS, other immunosuppression	Limited influence on cutaneous leishmaniasis. Susceptibility to visceral leishmaniasis increased, but not disease severity. More likely to relapse after treatment.	Herwaldt, 1999
American trypanosomiasis (Chagas disease)	AIDS, lymphoblastic lymphoma, cardiac transplantation, other immunosuppression	In AIDS, central nervous system often involved, myocarditis, skin lesions.	John & Petri, 2006; La Hoz et al., 2019
Babesiosis	Splenectomy	Usually subclinical infection in those with intact host defenses. Splenectomized patients usually develop clinically evident disease, which can be fatal.	Westblade et al., 2017
Helminth			
Strongyloidiasis	Immunosuppression for transplantation or by cancer chemotherapy or adrenal corticosteroids or lymphoma. AIDS is not a major predisposing factor	Because of endogenous autoinfection, hyperinfection or disseminated infection can develop and present as pneumonia or severe intestinal disease. Gram-negative sepsis or gram-negative meningitis can result from translocation of intestinal bacteria via invading larvae. Should check for strongyloidiasis before immunosuppressing patient from highly endemic areas.	Hayes & Nellore, 2018
Arthropod			
Scabies	Malignancy, transplant immunosuppression, antineoplastic therapy	AIDS leads to "Norwegian" or crusted scabies with widespread involvement by thick, crusted lesions. Sometimes has severe itching. Patient has numerous mites and is therefore highly contagious. Often less responsive to therapy.	Mullen & Durden, 2018; Sandoval et al., 2018

AIDS, Acquired immunodeficiency syndrome.

species are capable of squirting these fluids over a distance of several centimeters. Exposure of the skin or mucous membranes to these fluids may produce a burning sensation and blister formation. Millipedes may also serve as intermediate hosts for *Macracanthorhynchus ingens*, a thorny-headed worm that causes zoonotic infections in humans.

Centipedes

Centipedes are flatter than millipedes, have only one pair of legs per body segment and longer antennae. They are fast moving and can inflict a painful sting from a pair of forward-directed pincers that are modified from the first pair of legs. Although they are rarely responsible for serious injury to humans, the larger species (26–45 cm) found in southern United States and in tropical regions are able to penetrate human skin when handled, giving a painful, burning sting with local tissue reaction. Although systemic reactions may occur in individuals who have been previously sensitized, fatalities are rare (Moraru & Goddard, 2019).

Crustaceans

Crustaceans of medical importance are primarily those species that serve as hosts for larval stages of several different helminths. Several genera of crabs and crayfish are intermediate hosts for the metacercariae of various species of lung fluke (*Paragonimus* spp.) found around the world. Copepods are common microscopic zooplankton, certain species of which serve as first intermediate hosts for the nematodes *D. medinensis* and *Gnathostoma spinigerum* and for cestodes of the genera *Dibothriocephalus*, *Adenocephalus*, *Diphyllobothrium*, and *Spirometra*.

Pentastomes, or tongue worms, are a highly modified group of parasitic crustaceans. Adult stages are wormlike organisms that live in the nasal passages of certain predatory reptiles, birds, and mammals. Larval stages

resemble mites and reside in rodents, herbivores, and freshwater fish. Human liver and lung infections with larval stages of *Armillifer* spp. have been reported from Asia and Africa. Adult stages of *Linguatula serrata* have been recovered from the nasopharynx of individuals from the Middle East and Africa, where they are responsible for an obstructive condition known as *halzoun* (Tappe & Buttner, 2009).

PARASITIC INFECTIONS AND THE IMMUNOCOMPROMISED HOST

Immunocompromised hosts have abnormalities in their humoral or cellular immune systems resulting from disease, therapy, or congenital abnormality. Severe malnutrition may also compromise host defenses. In most instances, the predisposing host abnormalities are primarily of the cellular (T-cell) immune system and result from AIDS, malignancy, chemotherapy of malignancy, immunosuppression for transplantation, corticosteroid therapy, or a combination of these. The predisposing immune defect for giardiasis is with humoral immunity, and for babesiosis it is with splenectomy.

With the worldwide epidemic of HIV infection and AIDS being particularly severe in underdeveloped countries with a high incidence of parasitic infection such as malaria, schistosomiasis, ascariasis, amebiasis, and filariasis, it is fortunate that these infections do not cause particularly severe disease in AIDS patients. However, as comorbidities, their contribution to health care costs is enormous.

Some parasitic infections—such as cryptosporidiosis, toxoplasmosis, and strongyloidiasis—are more severe in immunocompromised hosts. However, there are differences depending on the type of immunosuppression. For example, although cryptosporidiosis and toxoplasmosis are particularly problematic in AIDS patients, strongyloidiasis is not a major problem in this population. However, it is a problem in transplant recipients, patients with HTLV-1 infection, and individuals receiving antineoplastic chemotherapy.

Table 65.12 lists parasitic infections that are more severe and/or frequent in immunocompromised patients. Clinical manifestations may be different from those seen in the general population, as is noted in the comment section.

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CHAPTER 66

SPECIMEN COLLECTION AND HANDLING FOR DIAGNOSIS OF INFECTIOUS DISEASES

Anna R. Plourde, Kathleen G. Beavis

KEY POINTS

- Collect specimens from site of infection before initiating therapy.
- Collect an adequate volume of sample for testing required.
- Tissue, fluid, or aspirates are always superior to a swab specimen. The only exception is collecting culture material from a hard-to-reach spot such as a throat or cervix.
- Use required collection and transport materials to preserve specimen integrity.
- Communicate clear orders and source information.
- Expedite the transport of specimens to the laboratory and do not allow them to sit in collection areas.

Appropriate specimen collection, transport, and processing are crucial preanalytical steps in the accurate diagnosis of infectious diseases. Guidelines for specimen handling are discussed in this chapter. General principles are reviewed first followed by discussion of the most common types of specimens submitted to the clinical microbiology laboratory for testing.

GENERAL PRINCIPLES

TIMING OF SPECIMEN COLLECTION

For optimal detection of the pathogens responsible for an infectious disease, specimens should be collected at a time when the likelihood of recovering the suspected agent is greatest. For example, the likelihood of recovering most viruses is greatest in the acute phase of the illness. Specimens for recovery of bacteria should ideally be collected before antimicrobial therapy is started.

SPECIMEN VOLUME

The volume of specimen collected must be adequate for performance of the microbiological studies requested. If insufficient volume is received, the health care worker caring for the patient should be notified; either an additional sample can be obtained, or the physician must prioritize the requests.

Very little specimen is obtained with a swab, and much of the specimen is retained within the swab tip. Swabs should not be used as collection devices unless the specimen source is the throat, cervix, or other difficult-to-reach area. If a swab is used to collect the specimen, a polyester-tipped swab on a plastic shaft is acceptable for most organisms. Calcium alginate should be avoided for collection of samples for viral culture because it could inactivate herpes simplex virus (HSV), cotton may be toxic to *Neisseria gonorrhoeae*, and wooden shafts should be avoided because the wood may be toxic to *Chlamydia trachomatis*. Swabs are not optimal for detection of anaerobes, mycobacteria, or fungi, and they should not be used when these organisms are suspected. An actual tissue sample or fluid aspirate is always superior to a swab specimen for the recovery of pathogenic organisms.

SPECIMEN COLLECTION

Specimens should be obtained from the site of infection with minimal contamination from adjacent tissues and organ secretions, and with the exception of stool, should be collected in a sterile container. All specimens should be labeled with the name and identification number of the person from whom the specimen was collected, the source of the specimen, and the date and time it was collected.

SPECIMEN TRANSPORT

After collection, specimens should be placed in a biohazard bag and transported to the laboratory as soon as possible. If a delay is unavoidable, urine,

sputum and other respiratory specimens, stool, and specimens for detection of *C. trachomatis* or viruses should be refrigerated to prevent overgrowth of normal flora. Cerebrospinal fluid (CSF) and other body fluids, blood, and specimens collected for recovery of *N. gonorrhoeae* should be held at room temperature because refrigeration adversely affects recovery of potential pathogens from these sources.

UNACCEPTABLE SPECIMENS

Each laboratory director must establish criteria for rejecting specimens unsuitable for culture. Most clinical microbiologists agree that the following specimens should be rejected:

- Any specimen received in formalin
- 24-hour sputum collections
- Specimens in containers from which the sample has leaked
- Specimens that have been inoculated onto agar plates that have dried out or are outdated
- Specimens contaminated with barium, chemical dyes, or oily chemicals
- Foley catheter tips
- Duplicate specimens (except blood cultures) received in a 24-hour period
- Blood catheter tips submitted for patients without concomitant positive blood culture

The following specimens should be rejected for anaerobic culture:

- Gastric washings
- Urine other than suprapubic aspirate
- Stool (except for recovery of *Clostridium difficile* for epidemiologic studies or for diagnosis of bacteria associated with food poisoning)
- Oropharyngeal specimens except deep tissue samples obtained during a surgical procedure
- Sputum
- Swabs of ileostomy or colostomy sites
- Superficial skin specimens

STANDARD PRECAUTIONS

Safety is the responsibility of the laboratory director and, per the Clinical Laboratory Improvement Amendments (CLIA), cannot be delegated to others. The laboratory director should collaborate with infection control, institutional safety committees, environmental services, engineering, and others within the hospital and department to ensure that there are current and necessary policies and procedures, engineering controls, personal protective equipment (PPE), and a trained work force. The laboratory director is also responsible for making sure the policies and procedures are followed. Specimen processing can have additional challenges because this workforce often has the least amount of formal technical education.

Universal Precautions were designed to protect workers from infectious substances in blood and body fluids. Body Substance Isolation Precautions were designed to protect workers from transmission of organisms from moist body surfaces. In 1996, the US Hospital Infection Control Practices Advisory Committee unified these into "Standard Precautions." As described in *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), Standard Precautions apply to (1) blood; (2) all body fluids, secretions, and excretions except sweat regardless of whether or not they contain visible blood; (3) nonintact skin; and (4) mucous membranes (Wilson, 2009). Standard Precautions must be followed when handling all specimens. A risk assessment should be performed in each laboratory area; it should detail the particular risks with each procedure. The safety recommendations for engineering controls, PPE, and work practices can be tailored to the risk (Callihan et al., 2014). Appropriate barriers are used to prevent exposure of skin and mucous membranes to the specimen. Gloves and a lab coat must be worn at all times when handling patient specimens, and masks, goggles (or working behind a plastic shield), and impermeable gowns or aprons must be worn when there is a risk for splashes or droplet formation. Optimally, all specimen containers, but at a minimum, those containing respiratory secretions and those submitted specifically for detection of mycobacteria or fungi should be opened in a biological safety cabinet. Specimens collected for virus isolation should be handled in a biological safety cabinet to prevent contamination of the cell cultures.

REFERRAL TESTING

When specimens or cultures must be shipped to a reference laboratory, they must be packaged according to dangerous goods shipping guidelines (see International Air Transport Association website, available at http://www.iata.org/whatwedo/cargo/dgr/Pages/index.aspx). Specimens must be limited to no more than 40 mL. Cultures of bacteria and fungi should be grown on solid media in tubes. The cap of the primary container (tube or vial) should be sealed with waterproof tape and inserted into a second container surrounded by sufficient packing material to absorb the entire volume of the culture or specimen if the primary container were to leak or break. If several primary tubes are placed in a second container, they must be either individually wrapped or separated so as to prevent contact between them, and there must be secondary packaging, which must be leakproof. The second container should be capped and placed in a shipping container made of corrugated fiberboard or hard plastic. An itemized list of contents must be enclosed between the secondary and outer packaging. The secondary and outer containers should be of sufficient strength to maintain their integrity at temperature and air pressures to which they will be subjected. If a specimen must be shipped on dry ice (which is considered to be a hazardous material), it must be marked "Dry ice, frozen medical specimen." The dry ice should be placed outside the second container with the packing material in such a way that the container does not become loose inside the outer container as the dry ice evaporates. All infectious shipping packages must be labeled with an official label containing the address and contents as well as the name and telephone number of the person responsible for the shipment. All laboratorians who package and ship materials that are known or reasonably expected to contain a pathogen must have documentation of training. There are several commercial and government-based resources for training.

BLOOD

The rapid identification and susceptibility testing of bloodborne pathogens is one of the most critical functions of the microbiology laboratory. Even with the increased use of molecular technologies capable of identifying organisms and some markers of resistance from either blood specimens or positive blood culture bottles, blood cultures are still the "gold standard" for identifying bacteria responsible for bacteremia, sepsis, infections of native and prosthetic valves, suppurative thrombophlebitis, mycotic aneurysms, and infections of vascular grafts. Blood cultures also are useful in diagnosing invasive or disseminated infections caused by certain fungi, especially *Cryptococcus neoformans*, *Candida* spp., *Fusarium* spp., and *Histoplasma capsulatum*. Serologic testing or nucleic acid amplification tests (NAATs) are needed to identify two important bacterial causes of culture-negative endocarditis, *Coxiella burnetii* and *Bartonella* spp. Parasites are usually detected in blood by microscopic examination of peripheral smears, but enzyme immunoassay (EIA) and NAATs are also used.

SPECIMEN COLLECTION

Timely detection and accurate identification of organisms in the blood depend on appropriate collection, transport, and processing of the specimen. In general, two to three sets of blood cultures should be collected for identification and susceptibility testing before antimicrobial therapy is initiated. Consensus guidelines recommend peripheral venipuncture rather than draws from intravascular catheters. Good skin cleaning and phlebotomy technique minimize the presence of skin contaminants in blood cultures (Miller et al., 2018). The use of iodine tincture, or chlorhexidine gluconate, is preferable to povidone–iodine solutions because the former require only about 30 seconds after application to achieve antiseptic effect; the latter requires up to 2 minutes. Each set of peripheral blood cultures must be drawn from a separate venipuncture site so that if a typical contaminating organism is present in one set and not the other, the organism can be more easily classified as a contaminant.

Compared with venipuncture, the risk for contamination is increased when blood cultures are drawn from an indwelling vascular device. Although it might seem advantageous to save the patient the discomfort of a venipuncture, a contaminated blood culture can cause even more diagnostic cultures, unnecessary antibiotics, and a prolonged stay in the hospital. If it is necessary to draw a culture through an intravenous line, another blood culture should be collected simultaneously from a venipuncture to aid in the interpretation of a contaminated line culture (Miller et al., 2018).

SPECIMEN TIMING AND VOLUME

The optimal time to draw blood for cultures when bacteremia or fungemia is suspected is just before a fever or chill, but this is not predictable. The single most important factor to detect bacteremia is volume of blood collected. In adults with bacteremia, the number of colony-forming units (CFUs) per milliliter of blood is frequently low. Therefore, for adults, collecting multiple sets of blood cultures with each bottle filled to optimal

volume is most important. (Washington & Ilstrup, 1986). In infants and children, the concentration of microorganisms in blood is higher, and collection of 1 to 5 mL of blood per culture is adequate. When blood cultures are drawn by personnel other than phlebotomists, it can be a challenge to obtain sufficient volumes in the culture bottles. With the current focus on detecting and treating sepsis, it behooves the pathologist to collaborate with clinical colleagues to optimize the amount of blood collected and improve the ability of the blood culture to detect bacteremia (Hazen et al., 2020; Khare et al., 2020).

SPECIMEN DRAWS

Recommendations concerning the number of blood specimens to collect are based on the nature of the bacteremia: transient, intermittent, or continuous. Transient bacteremia follows manipulation of a focus of infection (e.g., an abscess, a furuncle, or cellulitis), instrumentation of a contaminated mucosal surface (as occurs during dental procedures, cystoscopy, urethral catheterization, suction abortion, or sigmoidoscopy), or a surgical procedure in a contaminated site (e.g., transurethral resection of the prostate, vaginal hysterectomy, colon resection, and debridement of infected burns). Transient bacteremia also occurs early in the course of many systemic and localized infections such as meningitis, pneumonia, pyogenic arthritis, and osteomyelitis. Most intermittent bacteremias are associated with an undrained abscess, whereas continuous bacteremia is the hallmark of intravascular infection, such as bacterial endocarditis, mycotic aneurysm, or an infected intravascular catheter. Continuous bacteremia also occurs during the first few weeks of typhoid fever and brucellosis.

The optimal number of blood cultures for detection of bacteremia in patients without endocarditis is controversial. Most authorities agree that two or three 20-mL blood samples drawn over a 24-hour period and equally distributed into aerobic and anaerobic blood culture bottles will detect most bloodstream infections. One investigator demonstrated that 80% of bacteremias were detected with two blood cultures and 96% with three blood cultures. All bacteremias were detected with four blood cultures, but the routine collection of four blood cultures (up to 80 mL of blood) should be weighed against the risk for anemia (Cockerill et al., 2004). The optimal time interval between cultures is unknown, but 30 to 60 minutes for the first two sets has been suggested, with another one to two sets drawn over the remaining 24 hours if symptoms of septicemia persist (Cockerill et al., 2004). However, if initiation of antimicrobial therapy is deemed urgent, cultures should be collected before therapy is begun, from separate sites, within a few minutes.

Organisms such as the coagulase-negative staphylococci, viridans streptococci, *Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium* spp. are frequent blood culture contaminants but may also be true pathogens. Collecting two sets of blood cultures per febrile episode helps distinguish probable pathogens from contaminants. If two sets are drawn from different venipuncture sites, the odds of both sets being contaminated by skin flora are very low. If two sets are drawn at the same time and only one set contains a skin contaminant, it is safe to assume that the culture was contaminated during collection. If only one set is drawn and a contaminant is present, it can be difficult to *not* treat the organism, especially if the patient has had recent surgery.

RECOVERY OF MICROORGANISMS

Host factors such as antibodies, complement, phagocytic white blood cells, and antimicrobial agents may impede recovery of microorganisms from blood; therefore, various approaches have been used to counteract these factors. Diluting the blood specimen in broth medium in a 1:10 ratio provides optimal neutralization of the serum bactericidal activity (Washington & Ilstrup, 1986). Incorporating 0.02% to 0.05% sodium polyanethol sulfonate in the blood culture medium inhibits coagulation, phagocytosis, and complement activation and inactivates aminoglycosides. Methods that counteract the presence of antimicrobial agents include using antibiotic-adsorbent resins or the lysis-centrifugation system.

AUTOMATED BLOOD CULTURE SYSTEMS

Three commercially available automated blood culture systems, each with advantages and disadvantages, are available. These continuously monitored automated detection systems have essentially replaced manual systems. All systems use nutritionally enriched liquid media, which are capable of supporting growth of most bacteria and many pathogenic yeasts. Traditionally, two bottles, an aerobic and an anaerobic, are inoculated.

Three major automated continuously monitoring blood culture systems are available commercially in the United States. With these systems, the usual incubation period is 5 days (Reisner & Woods, 1999). The BacT/ALERT 3D system (bioMerieux) is based on the colorimetric detection of carbon dioxide (CO_2) produced during microbial growth. A CO_2 sensor is bonded to the bottom of each blood culture bottle and is separated from the broth medium by a membrane that is impermeable to most ions and to components of media and blood but freely permeable to CO_2. Inoculated bottles are placed in cells in the instrument, which provides continuous rocking of both aerobic and anaerobic bottles. If bacteria are present, they generate CO_2, which is released into the broth medium; the pH then decreases, causing the sensor to change color from green to yellow. Color changes are monitored once every 10 minutes by a colorimetric detector. This system supports the growth of aerobic and anaerobic bacteria; bottles are available to support the growth of mycobacteria. This system has received Food and Drug Administration (FDA) clearance for monitoring bacterial contamination of platelets.

The BACTEC continuous-monitoring system (BD Diagnostics) is based on fluorescent technology. Bonded to the base of each vial is a CO_2 sensor that is impermeable to ions, medium components, and blood but freely permeable to CO_2. If organisms are present, they release CO_2 into the medium; it then diffuses into the sensor matrix and generates hydrogen ions. The subsequent decrease in pH increases the fluorescence output of the sensor, changing the signal transmitted to the optical and electronic components of the instrument. The computer generates growth curves, and data are analyzed according to growth algorithms. Inoculated bottles are placed in individual cells of the instrument and rocked continuously. Adult and pediatric aerobic and anaerobic bottles are available, as well as a bottle for the recovery of yeasts and mycobacteria.

The VersaTREK system (Trek Diagnostic Systems) detects growth of organisms in broth by measuring gas consumption and gas production. Each inoculated vial is fitted with a disposable connector that contains a recessed needle. The needle penetrates the bottle stopper and connects the bottle headspace to the sensor probe. The sensor monitors changes within the headspace in the consumption and production of all gases (CO_2, N_2, and H_2) by growing organisms and creates data points internally in the computer. Media are available to identify aerobic and anaerobic bacteria, including mycobacteria. Some clinicians continue to request extended incubation protocols for *Brucella* spp. and other bacteria known as the HACEK group (*Haemophilus*, *Aggregatibacter* [formerly *Actinobacillus*], *Cardiobacterium*, *Eikenella*, *Kingella* spp.). These organisms needed prolonged incubation times when manual methods predominated, but they can be detected during the routine incubation times of the automated instruments.

When mycobacteria or fungi are suspected, liquid medium developed by the manufacturer of automated and semiautomated broth culture systems can be used. These blood cultures are incubated for a prolonged period of 4 to 8 weeks.

DETECTION AND NOTIFICATION OF POSITIVE CULTURES

Positive blood cultures containing commonly isolated aerobic organisms are usually detected within 12 to 36 hours of incubation. Until recently, the initial report was limited to a Gram stain; identification and susceptibility results could be expected no sooner than 24 to 48 hours after the Gram stain report. Both the FilmArray blood culture identification panel (BioFire Diagnostics) and the Verigene system (Nanosphere) use molecular methods to identify more than 90% of the organisms in the time when a Gram stain would be reported. They can also identify the presence or absence of *mecA* and other resistance genes, allowing empiric therapy to be tailored (Bhatti et al., 2014a). The Accelerate Pheno system (Accelerate Diagnostics) can identify the organisms causing 90% of bacteremias and provide minimal inhibitory concentrations of key antimicrobial agents within 8 hours of a positive blood culture (Charnot-Katsikas et al., 2018). As these systems evolve and continue to provide even more rapid, clinically actionable information, laboratories should work with clinical colleagues to make sure these more expensive modalities have impact on patient care. "Pushing" information to clinicians has been shown to decrease the time to escalation or de-escalation of empiric antimicrobials (Banerjee et al., 2015).

The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has shortened the time to identification. Although most laboratories allow for overnight incubation before testing mature colonies, an aliquot of the positive culture can be centrifuged and the pellet inoculated onto prewarmed plates. About 95% of isolates can be identified with the MALDI-TOF after 4 hours of incubation (Bhatti et al., 2014b). Although more labor intensive, the reagents of

this method are much less expensive than the molecular methods. Cultures containing anaerobes are usually not detected for 48 to 72 hours, and identification is not available for 3 to 4 days. Fastidious organisms, such as those found in the HACEK group, may not be detected until 3 to 5 days.

In a small percentage of cases, no organisms will be seen on a Gram stain from a positive blood culture. It is very important to inoculate and monitor media in these cases. *Brucella* spp. can be difficult for an inexperienced eye to note on Gram stain; the colonies are visible on solid media a few days later. Conversely, some organisms are visible on Gram stain but not on routine media. These organisms grow on chocolate agar or sheep blood agar with a *Staphylococcus aureus* streak.

DETECTION OF VIRUSES

With regard to viruses, blood specimens are most commonly collected to monitor response of infection with human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), or cytomegalovirus (CMV) to antiviral therapy by using quantitative polymerase chain reaction (PCR) to measure viral load. Such assays are commercially available for each of these viruses, and in all cases, manufacturer's guidelines for specimen collection and transport should be followed. For HIV and HCV, blood specimens also may be collected for genotyping (commercial assays are available), and PCR (qualitative or quantitative PCR) generally is used to confirm an initial positive HCV antibody result. As with viral load, manufacturer's guidelines regarding specimen collection and transport should be followed.

In addition to assessing response to antiviral therapy, measuring viral load in a blood specimen is useful for monitoring disease and for diagnosis of disease in specific situations. In immunocompromised patients, especially transplant recipients but also patients with acquired immunodeficiency syndrome (AIDS), determining the level of CMV deoxyribonucleic acid (DNA) in blood is used to predict those at high risk for development of CMV disease and direct the initiation of preemptive therapy. Monitoring the level of BK virus and Epstein-Barr virus (EBV) DNA in serum or plasma by quantitative PCR is indicated in transplant recipients. If a commercially available NAAT assay is used, the package insert guidelines for specimen collection and transport should be followed. If, on the other hand, an assay developed and validated in house is used, guidelines published by that laboratory should be followed.

DETECTION OF PARASITES

Blood specimens are useful for diagnosis of malaria, babesiosis, trypanosomiasis, and some filariasis (Rosenblatt, 2009). Specimens should be collected in tubes with anticoagulant and transported promptly to the laboratory. If smears must be sent to a reference laboratory, they should follow the reference laboratory's instructions for fixation soon after they are made. The techniques used in the laboratory for detecting the aforementioned parasites are the same and are discussed here in order of the simplest to the most complicated.

Standard Precautions need to be used when preparing smears and reading fresh (unfixed) smears. Additional fixation time in methanol could be recommended depending on the patient's travel history. The simplest technique for detecting parasites in a sample of blood is the direct mount, prepared by placing 1 drop of blood on a glass slide, covering it with a cover glass, and examining it immediately. Direct mounts are excellent for diagnosis of trypanosomiasis or filariasis because the trypomastigotes and the microfilariae easily can be seen moving, often with low or medium power. Stained smears make the definitive diagnosis.

The thin smear, made as for hematologic work and stained in a similar manner, is the standard preparation for speciating *Plasmodium* spp., *Babesia* spp., *Trypanosoma* spp., and microfilaria. Thin smears for parasitologic work are fixed and then preferably stained manually with Giemsa stain, but automated hematologic staining is adequate. Smears are first scanned at low power to detect microfilariae, which are large objects (between 100 and 200 μm) and easily seen, usually at the lateral edges of the smear. After they are located, microfilariae should be studied under oil immersion for identification. After scanning with low power, the smear is examined with a high dry objective, searching for trypanosomes, and finally under oil immersion to find and identify *Plasmodium*, *Babesia*, and *Trypanosoma* spp.

Thick smears are useful for detecting all the parasites mentioned earlier and are part of the minimum laboratory workup for their diagnosis. One drop of blood is placed on a clean glass slide and, with the corner of another slide, is gently spread to cover 1 cm square. The preparation is allowed to dry and without fixation is stained with Giemsa stain, allowing for its dehemoglobinization.

BODY FLUIDS

CEREBROSPINAL FLUID

CSF is collected to diagnose meningitis and, less frequently, viral encephalitis. Infectious meningitis, a medical emergency requiring early therapy to prevent death or serious neurologic sequelae, is divided into acute, subacute, and chronic clinical syndromes, based on duration of symptoms. Potential pathogens are listed in Table 66.1. Enteroviruses are the agents most commonly responsible for meningitis, and they should be considered first in the differential diagnosis of meningitis in a child or adolescent during the late summer and early fall. The pyogenic bacteria responsible for meningitis vary with the age of the affected individual (Table 66.2).

Sample Collection and Transport

CSF is usually obtained by lumbar spinal puncture, but sometimes it is aspirated from the ventricles or collected from a shunt. As when collecting blood for culture, careful skin antisepsis is essential for collection of CSF, which typically is submitted to the laboratory in three or occasionally four tubes. The first tube filled should not be sent to Microbiology because it can contain skin flora. At least 0.5 to 1.0 mL should be sent to Microbiology for Gram stain and bacterial culture; ideally, the microbiology laboratory would receive 5 to 10 mL to accommodate requests for NAAT and cultures for mycobacteria and fungi (Miller et al., 2018). The parameters of normal CSF and the usual changes that occur during meningitis caused by different organisms are listed in Table 66.3.

CSF should be transported promptly to the laboratory and processed as rapidly as possible. If a brief delay in processing is unavoidable, the specimen should be held at room temperature unless viral culture is requested, in which case a portion (preferably 1 mL but no less than 0.5 mL) may be refrigerated for a short time. Specimen processing differs for bacteria, fungi, viruses, and parasites and is discussed separately for each group of organisms.

TABLE 66.1
Infectious Meningitis Syndromes

Syndrome	Onset or Duration	Probable Pathogens
Acute	<24 hours	Pyogenic bacteria
Subacute	1–7 days	Enteroviruses, pyogenic bacteria
Chronic	Persisting at least 4 weeks	*Mycobacterium tuberculosis* *Treponema pallidum* *Brucella* spp. *Leptospira interrogans* *Borrelia burgdorferi* *Cryptococcus neoformans* *Coccidioides immitis* *Histoplasma capsulatum*

TABLE 66.2
Common Bacterial Causes of Acute Meningitis by Age

Age	Organisms
Neonates–3 months	Group B streptococcus *Escherichia coli* *Listeria monocytogenes*[*] *Streptococcus pneumoniae*
4 months–6 years[†]	*Streptococcus pneumoniae*
6–45 years	*Neisseria meningitidis*
Older than 45 years	*Streptococcus pneumoniae* *Listeria monocytogenes* Group B streptococcus

[*]May cause meningitis in immunocompromised individuals in all age groups.
[†]Incidence of meningitis caused by *Haemophilus influenzae* type b in the United States has declined dramatically as a result of vaccination.

TABLE 66.3
Normal Cerebrospinal Fluid Parameters and Changes in Infectious Meningitis

Condition	WBCs (cells/μL)[a]	Protein (mg/dL)	Glucose (mg/dL)
Normal	5 (lymphocytes)	14–45	45–100 (2/3 serum)
Meningitis			
Acute or subacute bacterial	>500 (PMNs)	↑	↓
Chronic bacterial	200–2000	↑	↓
Tuberculous, fungal	(lymphocytes)	↑	↓
Enteroviral	200–2000 (PMNs early; lymphocytes later)	↑	Normal

[a]Cell type listed usually predominates.
↑, Increased; ↓, decreased; *PMN,* polymorphonuclear leukocyte; *WBC,* white blood cell.

Sample Processing for Bacterial and Fungal Culture

Processing CSF for routine bacterial culture includes concentration (if 1 mL or more of specimen is received), preparation of a smear by cytocentrifugation for staining with Gram stain, and culture. The supernatant is decanted into a sterile tube, leaving about 0.5 mL of sediment and fluid, which is thoroughly mixed on a vortex mixer or by forcefully aspirating up and down into a sterile pipette.

Diagnosis of chronic bacterial meningitis requires specific requests because the CSF is handled differently for each entity. To diagnose brucellosis, the CSF is processed as described earlier for routine bacterial culture, but the media are incubated for 2 to 3 weeks. For leptospirosis, *Leptospira interrogans* may be cultured from the CSF during the first few weeks of illness, but the Centers for Disease Control and Prevention (CDC) can test using a NAAT. The diagnosis of neurosyphilis can be challenging and requires neurologic signs and symptoms, reactive serologic results, and CSF findings including pleocytosis, elevated protein, and a positive Venereal Disease Research Laboratory (VDRL) test result. If the CSF-VDRL result is negative in the presence of clinical and serologic support for neurosyphilis, a CSF fluorescent treponemal antibody absorption test might be useful. This test is more sensitive and less specific that the CSF-VDRL (https://www.cdc.gov/std/tg2015/syphilis.htm). The specimen should be refrigerated until it is tested. Involvement of the central nervous system by *Borrelia burgdorferi* (Lyme disease) also is diagnosed serologically by detection of specific immunoglobulin (Ig) M and IgG antibodies in CSF and serum.

At least 5 mL of CSF is recommended for mycobacterial culture; cytocentrifugation is required to make a smear and optimize the yield. A NAAT may be useful, but a negative result does not exclude mycobacterial infection.

Processing CSF for detection of fungi is similar to that described for detecting bacteria. Organisms are concentrated by filtration or by centrifugation. A cytocentrifuge preparation or a smear of the sediment stained with Gram or other stain is examined, and appropriate media (e.g., brain-heart infusion or SABHI agar without antibiotics) are inoculated for culture.

Additional Diagnostic Tests

In addition to culture, there is a commercially available molecular panel for meningitis including bacterial, viral, and fungal targets. This panel does not replace culture; the panel does not detect all CSF pathogens, and culture is still necessary for susceptibility results (Liesman et al., 2018; Tansarli & Chapin, 2020).

Two types of rapid tests are available for diagnosis of meningitis caused by *C. neoformans:* those specific for the capsular antigen (latex agglutination and enzyme-linked immunosorbent assay [ELISA]) and the nonspecific India ink preparation, which allow visualization of encapsulated yeast cells (Fig. 66.1). The sensitivity of the India ink stain, performed by mixing 1 drop of CSF sediment with 1 drop of India ink (available at art supply stores), is low, except in HIV-infected persons. Therefore, the cryptococcal latex agglutination test or the ELISA, both of which are highly specific and have sensitivities of more than 90%, is recommended for diagnosis.

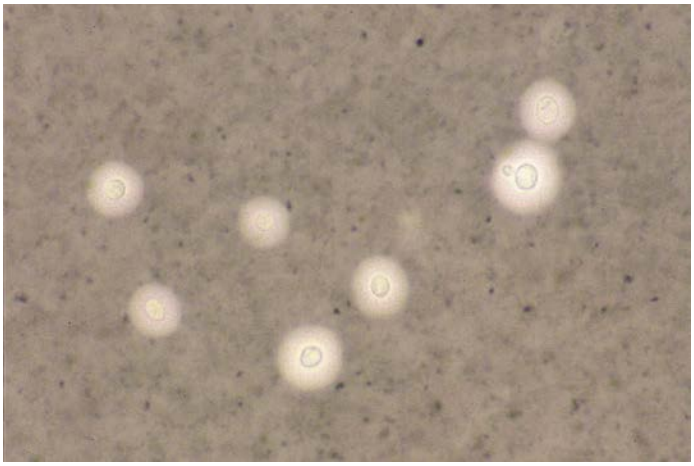

Figure 66.1 India ink preparation of cerebrospinal fluid showing encapsulated yeast forms of *Cryptococcus neoformans.* (400×.)

Supernatant of a centrifuged specimen or unspun CSF can be used for these latter two tests.

Cerebrospinal Fluid Sample Processing for Diagnosis of Viral and Parasite Infections

Currently, NAATs are used most often for diagnosis of viral infections of the central nervous system. Other diagnostic methods are conventional cell culture (primarily for detection of enteroviruses, although PCR is preferred) and serologic tests for viruses that cause encephalitis (western equine, eastern equine, Venezuelan equine, St. Louis, Japanese, La Crosse, and West Nile viruses).

CSF is occasionally sent to the laboratory for diagnosis of African trypanosomiasis (*Trypanosoma gambiense* and *Trypanosoma rhodesiense*) or infection with free-living amebae (*Naegleria fowleri* and *Acanthamoeba* spp.). When the specimen is received in the laboratory, it should be processed immediately. Wet preparations are prepared directly from the specimen and from the sediment by first shaking the tube gently (a step necessary because the parasites often stick to the wall of the tube) and then centrifuging the specimen at 250 *g* for 10 minutes. Preparations are examined under the microscope with the condenser in a low position to allow visualization of trophozoites or, preferably, by phase contrast microscopy.

Cultures of free-living amebae from CSF are done on nonnutrient agar plates covered with a suspension of *Escherichia coli* or *Enterobacter aerogenes*. The fluid is centrifuged at 250 *g* for 10 minutes, the supernatant is removed with a sterile pipette, and the sediment is mixed with 0.5 mL of saline solution and poured at the center of the plate. The culture is incubated at 37°C and examined for amebae daily for 10 days using a microscope under a 10× objective (Martinez & Visvesvara, 1991).

OTHER BODY FLUIDS

Fluid is collected from the pericardial, thoracic, or peritoneal cavity or from joint spaces by aspirating with a needle and syringe. A volume of 1 to 5 mL is adequate for isolating most bacteria, but 10 to 15 mL is optimal for recovery of mycobacteria and fungi, which are generally present in low numbers. Moreover, to diagnose peritonitis associated with chronic ambulatory peritoneal dialysis, collection of at least 50 mL of fluid may improve recovery of the responsible pathogen. To transport the fluid, it is aspirated into a sterile container and delivered promptly to the laboratory. Alternatively, peritoneal fluid may be directly inoculated into blood culture bottles at the patient's bedside; however, submission of fluid in blood culture bottles eliminates the possibility of direct Gram staining and delays the identification and susceptibility testing of any pathogens isolated. Clinicians should be advised to send fluid in a sterile container as well as in blood culture bottles to allow for the performance of a Gram stain and faster identification if the culture is positive.

Enteroviruses, primarily Coxsackie viruses A and B, are among the most common causes of infectious pericarditis. These viruses may be detected in pericardial fluid by NAAT or traditional cell culture, but because they are not recovered in all cases, collection of throat washings and stool (which are more likely to yield the virus), in addition to pericardial fluid, is strongly recommended for virus isolation from persons with suspected enteroviral pericarditis. Other viruses (HSV, varicella zoster virus [VZV], CMV, EBV, HBV, mumps virus, and influenza virus) are infrequent agents of pericarditis and usually are not detected in pericardial fluid.

Sample Processing for Bacterial Culture

Processing fluid from body cavities for detection of bacteria involves preparing a smear for Gram stain and inoculating appropriate media for culture. As mentioned earlier, the sample may be inoculated into blood culture bottles at the bedside, although this is not optimal unless additional specimen is sent for Gram stain and inoculation onto media. In the laboratory, the fluid is centrifuged, and the supernatant is removed, leaving about 0.5 mL of fluid in addition to the sediment, which is mixed thoroughly and then used to prepare smears and inoculate media. Alternatively, a small volume of noncloudy, nonviscous fluid (about 0.1 mL) may be removed before centrifugation and used to prepare a cytocentrifuged smear.

Fluid specimens submitted for detection of mycobacteria are processed as described earlier for CSF. Fluids for fungal culture should be concentrated by centrifugation as described for bacteria. The supernatant is removed, leaving 1.5 to 2.0 mL, in which the sediment is thoroughly mixed. A smear of the sediment is prepared for staining with Gram or Calcofluor white. Ideally, 0.5 to 1.0 mL of sediment is inoculated to primary fungal planting media (as for CSF), but lesser volumes are acceptable.

Parasitologic Examination

Body fluids are rarely collected for detection of parasites; however, *Entamoeba histolytica* may be found in the pericardial, pleural, or peritoneal cavity as a result of rupture of an abscess of the liver (into the peritoneal, pleural, or pericardial cavity) or of the lungs (into the pleural or pericardial cavity) or to perforation of amebic ulcers (into the peritoneal cavity). Hydatid cysts are infrequently diagnosed by examination of body cavity fluid, also caused by rupture of a cyst into a viscus contiguous to the cavity in question. The fluid collected is usually clear and contains hydatid sand (see Chapter 60) but rarely is turbid because of superimposed bacterial infection. Uncommonly, in individuals with a filarial infection, examination of wet preparations of a body cavity fluid may demonstrate the microfilariae; in patients with *Strongyloides* hyperinfection, larvae may be detected in body cavity fluids.

TISSUES

Tissue specimens obtained surgically are procured at great expense and at considerable risk to the patient; therefore, it behooves the surgeon to obtain an amount of material that is adequate for both histopathologic and microbiological examination. Swabs are rarely adequate for this purpose because of their limited capacity to hold specimen for culture. The histopathology of the lesion serves not only to differentiate between infection and malignancy but also to distinguish between a suppurative and a granulomatous process. In some cases, special stains are helpful in establishing the cause of the process. In chronic lesions, the differential diagnosis includes disease caused by actinomycetes, *Brucella* spp., mycobacteria, and fungi, any one of which may be present only in small numbers, again emphasizing the need to obtain adequate samples for examination and culture.

SPECIMEN COLLECTION AND PROCESSING

Tissue obtained surgically for culture should be placed into a sterile, wide-mouthed, screw-capped container. As a general rule, tissue should be bisected aseptically by the surgeon in the operating room, and material representative of the pathologic process should be submitted for both histopathologic and microbiological examination. Good communication between the anatomic pathologist and microbiologist is important, especially in cases of fever of unknown origin for which an exploratory laparotomy is being performed and multiple biopsy specimens are taken.

Tissue received in the laboratory is finely minced with sterile scissors or scalpels, added to a small volume of broth, and then rendered homogeneous either in a tissue grinder, mortar and pestle, or Stomacher to provide a 20% suspension. This suspension is used to inoculate all of the necessary culture media and is then stored under refrigeration for at least 2 weeks before being discarded.

Soft tissue specimens can be submitted after many injuries, including burns, animal or human bites, trauma, or surgery. The likely pathogens vary with the type of injury. When soft tissue specimens are received, there should be accompanying information to guide the workup in Microbiology. Human mouth flora is typically found in human bites; bites from animals are associated with organisms typical to the biting animal (e.g., *Pasteurella canis* from dogs and *Pasteurella multocida* from cats). Animal bites are typically polymicrobial and contain both aerobes and anaerobes. Soft tissue specimens after trauma can contain organisms depending on the type of trauma, including organisms usually thought of as environmental contaminants. Soft tissue specimens from burns can require quantitative cultures. These are best performed in concert with histopathologic examination to assess the true extent of the infection (Miller et al., 2018).

EYE

The most common ocular specimens received in clinical laboratories are conjunctival and corneal samples collected for the diagnosis of conjunctivitis and keratitis, respectively. Because specimen material is often scant, processing should be strategized based on the most likely etiologic organisms and informed by consultation with the clinician. The necessary collection materials, including culture media for direct inoculation or viral transport media (VTM), should be provided by the laboratory. Specimens should be labeled with the exact anatomic site (e.g., conjunctiva, cornea) to aid appropriate microbiological workup (Miller et al., 2018).

CONJUNCTIVAL SPECIMENS

Conjunctival scrapings or swab specimens are collected to determine the etiologic agent of conjunctivitis. Bacteria are the most common etiologic agents of infectious conjunctivitis, and those most frequently implicated are *Streptococcus pneumoniae* and *S. aureus* in adults; and *Haemophilus influenzae*, *S. pneumoniae*, and *S. aureus* in children. Trachoma, caused by the bacterium *C. trachomatis*, is a leading cause of blindness worldwide. *C. trachomatis* may also cause inclusion conjunctivitis in newborns and, less commonly, in adults. Viruses are responsible for about 15% to 20% of cases of acute infectious conjunctivitis, and in the United States, most epidemics of viral conjunctivitis (pinkeye) are caused by adenoviruses or herpes simplex virus. Rarely, parasites are causes of conjunctivitis.

Specimen Collection and Processing

Conjunctival cells are obtained from the superior and inferior tarsal conjunctiva by using a swab moistened with broth or a sterile platinum spatula. If a bacterial or fungal infection is suspected, culture media are inoculated directly by the individual collecting the sample to maximize the quantity and viability of organism recovered and the likelihood of detection. Media typically includes a chocolate agar plate for recovery of fastidious bacteria and fungi, with additional media as necessary based on suspected organisms (Das et al., 2010). Ideally, direct smears are also prepared, which may be useful in preliminary identification of the organism. If direct preparation of smears and inoculation of media is not possible, swab specimens may be obtained, though swabs yield minimal material. Smears should be air dried and promptly transported, with the inoculated media, to the laboratory. The conjunctiva and the eyelids of both the involved and uninvolved eye are often cultured concomitantly to characterize the normal flora, useful in assessing culture results from the involved eye.

If viral conjunctivitis is suspected, a second sample (swab or scrapings) should be collected and placed in viral transport medium. Viral conjunctivitis is now primarily diagnosed using NAATs because of their increased sensitivity and rapid turn-around time but require validation because there are no NAATs for any ocular source that are FDA approved to date. Viral culture remains an option if a NAAT is not available. A rapid diagnosis may also be provided by direct or indirect immunofluorescent staining of thin smears of conjunctival cells with virus-specific antibodies.

To detect *C. trachomatis*, NAATs are now the test of choice but, as for viral NAATs, require in-house validation (Dize et al., 2013). Alternative methods include examination of direct smear and culture. Specimens collected in viral or chlamydia transport medium are appropriate for all three types of methods (Miller et al., 2018). For direct examination, a smear prepared from conjunctival scrapings may be stained with Giemsa stain and examined for epithelial cells with basophilic intracytoplasmic inclusions diagnostic of *C. trachomatis* or preferably with monoclonal fluorescent antibodies, which are more sensitive and specific than Giemsa. The swab is rolled across the surface of the glass slide provided, the material is fixed, and the slide is transported promptly to the laboratory and held at room temperature or refrigerated briefly. Slides are stained and examined with a fluorescent microscope for elementary bodies (see Chapter 64). Specimens containing fewer than 10 columnar or metaplastic squamous cells are considered inadequate, and results should be reported as inconclusive, with an explanation, and another specimen should be requested. Culture of *C. trachomatis* should be performed when a diagnosis of chlamydial conjunctivitis is strongly suspected but other methods have produced negative results.

CORNEAL SPECIMENS

Corneal scrapings and biopsy specimens are useful in determining the etiologic agent of keratitis, an infection that can cause loss of vision and

requires immediate attention. Bacteria account for 65% to 90% of keratitis cases. In the United States, *S. aureus*, *S. pneumoniae*, *Pseudomonas aeruginosa*, and *Moraxella* spp. are most frequently implicated. Contamination of contact lens storage cases is a predisposing factor for keratitis; outbreaks have infrequently been caused by *Fusarium* spp. and *Acanthamoeba* spp. Viral keratitis is most commonly caused by recurrent HSV type 1, VZV, and EBV; the adenoviruses are less frequent causes. Postsurgical infections can be caused *S. aureus*, coagulase-negative staphylococci, and the *Mycobacterium chelonae–Mycobacterium abscessus* group. If keratitis follows a traumatic event, the laboratory should culture for environmental contaminants such as *Nocardia* spp., fungi, and *P. aeruginosa*, in addition to the organisms that often present as normal flora, such as *S. aureus*, coagulase-negative staphylococci, *S. pneumoniae*, and *Cutibacterium* spp (Gray et al., 2011).

Specimen Collection and Transport

Corneal scrapings are collected with a sterile platinum spatula and are used for preparation of smears by direct transfer to glass slides for staining and for inoculation to appropriate media for culture. Media typically include chocolate agar for recovery of fastidious bacteria, as well as brain–heart infusion agar with 10% sheep blood and inhibitory mold agar for fungal isolation (Leber, 2016). If evaluation for viral keratitis is requested, scrapings should be placed directly into VTM and delivered promptly to the laboratory. Viral keratitis is now diagnosed primarily with NAATs, though no FDA-approved assays are yet available. Viral culture remains an option if a NAAT is not available. Immunofluorescent staining of thin smears prepared directly from scrapings may be performed using virus-specific antibodies (Azher et al., 2017). When the culture of scrapings of a suspicious corneal ulcer is negative, a superficial keratectomy or corneal biopsy specimen may be obtained by the ophthalmologist, an approach especially useful for detection of fungi and *Acanthamoeba* spp.

RESPIRATORY TRACT

NASOPHARYNGEAL SPECIMENS

Nasopharyngeal aspirates, washings, and swab specimens are collected predominantly for diagnosis of viral respiratory infections, now typically diagnosed using rapid molecular methods (see Chapter 64). Nasopharyngeal specimens may also be submitted for workup of bacterial pneumonia caused by *Bordetella pertussis*, *C. trachomatis*, *Chlamydia pneumoniae*, or rarely *Corynebacterium diphtheriae* or to identify carriers of methicillin-resistant *S. aureus* (MRSA). Endoscopically or surgically collected specimens from the nasal sinuses are submitted for workup of sinusitis, typically in chronic or complicated cases, which are often associated with *S. aureus*, gram-negative bacilli, *Streptococcus* spp., anaerobes, or fungi (Miller et al., 2018).

Specimen Collection, Transport, and Processing

Nasopharyngeal aspirates and washings are superior to swabs for viral recovery, but swabs are frequently submitted because they are more convenient. Whereas washings or swab specimens are collected for detection of *B. pertussis*, swabs can be used for detection of *C. pneumoniae*. A swab is the preferred specimen for *C. trachomatis* and *C. diphtheriae*.

An aspirate is collected with a plastic tube (e.g., one used to feed premature infants) attached to a 1-mL syringe or a suction catheter with a mucus trap. A wash is obtained with a rubber suction bulb by instilling and withdrawing 3 to 7 mL of sterile phosphate-buffered saline. Swabs are obtained by removing all mucus from the nasal cavity, inserting a small flexible nasopharyngeal swab along the nasal septum to the posterior pharynx, and rotating against the mucosa several times. It is important to note that whereas cotton-tipped swabs and calcium alginate swabs inhibit PCR reactions, cotton-tipped swabs are toxic to *B. pertussis* (Cloud et al., 2002). Dacron or nylon swabs are preferable alternatives.

To detect viruses, nasopharyngeal specimens are placed into an appropriate transport medium, with or without antibiotics, and transported promptly to the laboratory or stored briefly in the refrigerator and packed in ice for transport as soon as possible. If a NAAT is used, the specimen must be collected as recommended by the manufacturer. Viral detection methods are discussed in more detail in Chapter 64.

The most sensitive method for detecting *B. pertussis* is PCR; a number of FDA-approved assays are commercially available (van der Zee et al., 2015; US FDA, 2020). If the sample must be transported to a reference laboratory for PCR, the swab should be shipped dry or in saline. To detect *B. pertussis* by culture, inoculation of washings or swab specimens at the bedside is optimal to preserve organism viability. If this is not possible, the sample is placed into sterile Casamino Acids broth, transported promptly to the laboratory, and processed within 2 hours for culture. If the sample must be sent to a reference laboratory for culture, the swab should be inoculated into a solid transport media such as Regan-Lowe or Bordet-Gengou agar, incubated at 37°C for 48 hours, and then shipped at ambient temperature. The direct fluorescent antibody (DFA) staining method, which provides a rapid diagnosis but is associated with false-positive and false-negative results, can be performed on a smear prepared from a nasopharyngeal swab or washing (van der Zee et al., 2015).

For detection of *C. trachomatis*, a nasopharyngeal swab specimen is collected with a polyester-tipped swab, which may be used for culture or for preparation of a smear for DFA staining. Detection methods are discussed in Chapter 62. For assessment of *C. trachomatis* or *C. pneumoniae*, the swab should be placed in an appropriate transport medium and transported to the laboratory as soon as possible or refrigerated for a short time. NAAT is recommended for detection of *C. pneumoniae* (Miller et al., 2018).

For culture of *C. diphtheriae*, swabbing multiple sites within the nasopharynx likely increases sensitivity. Any membranes present, as well as areas from beneath membranes, should be sampled for highest yield. Swabs should be immediately transferred to the microbiology laboratory. If delay is unavoidable, a semisolid transport medium (e.g., Amies) helps maintain bacterial viability (Efstratiou et al., 2000).

Carriers of MRSA may be detected within a few hours by commercial NAATs or after 24 to 48 hours using MRSA-specific chromogenic agar media (Bocher et al., 2008; Bischof et al., 2009; Tacconelli et al., 2009). Nasal secretions are collected from the anterior nares with a polyester-tipped swab, which is placed in a tube transport system and promptly delivered to the laboratory. If a NAAT is used, the specimen must be collected with the swab recommended by the manufacturer.

THROAT SPECIMENS

Throat swabs can be used to diagnose bacterial pharyngitis and Vincent's angina (caused by *Fusobacterium necrophorum* and other anaerobes). Group A streptococcus (GAS) is the cause of pharyngitis in less than one third of cases, but GAS needs to be identified and treated to prevent acute rheumatic fever, suppurative sequelae, transmission to others, and worsening of signs and symptoms. *C. diphtheriae* and *N. gonorrhoeae* are the other bacterial causes of pharyngitis for which antimicrobial therapy has been demonstrated to be effective. Other streptococci (group C and group G) also cause pharyngitis, but the utility of identifying them from culture is controversial. Antibiotics have been given to lessen the duration of symptoms, but controlled studies demonstrating evidence of a clinical response are lacking (Shulman et al., 2012).

Throat washings or swab specimens are also useful for detection of viruses shed in oral secretions without causing pharyngitis (HSV, CMV, or enteroviruses).

Because of the risk for respiratory obstruction, throat swabs are contraindicated for workup of epiglottitis (Rabinowitz, 1978).

Specimen Collection and Transport

Throat swab specimens are collected by depressing the tongue with a tongue blade, introducing the swab between the tonsillar pillars and behind the uvula without touching the lateral walls of the buccal cavity, and swabbing back and forth across the posterior pharynx. Swab specimens collected for detection of viruses should be placed in a viral transport medium, and those for detection of bacteria should be placed in a tube transport system containing modified Stuart's medium. For detection of *N. gonorrhoeae*, use of calcium alginate swabs should be avoided because they are toxic to the organism and can inhibit PCR (Lauer & Masters, 1988). Dacron-, rayon-, polyurethane-, and nylon-tipped swabs are acceptable alternatives. Specimens should be inoculated immediately to a selective medium, such as modified Thayer-Martin agar, or placed into charcoal-containing or other specific medium (Graver & Wade, 2004). Throat washings for diagnosis of viral infections are obtained by gargling with 5 mL of viral transport medium containing antibiotics. Throat washings and swab specimens should be delivered promptly to the laboratory or refrigerated for a short time if a delay in transport is unavoidable.

SPECIMEN PROCESSING

For diagnosis of GAS pharyngitis, culture is most sensitive; however, this requires overnight incubation. GAS grows well on horse and sheep blood agar, producing complete hemolysis (beta-hemolysis) of the agar. For rapid diagnosis of GAS, there are many commercially available point-of-care tests. These tests have a high specificity, so a throat culture does not need to follow a positive result. However, their sensitivity is lower. A commercial direct probe is the most sensitive of the rapid tests, but this test is not

usually used as a point-of-care test (Shulman et al., 2012). Other rapid, direct tests for GAS (several are commercially available) are less sensitive (as low as 70%); therefore, if one of these is used, two throat swab specimens should be collected. If the direct test result is positive, the second swab may be discarded, but if the direct test result is negative in children, a confirmatory culture must be performed using the second swab. In adults, because the incidence of streptococcal infection and the risk for rheumatic fever are low, confirmatory culture is optional (Shulman et al., 2012).

To detect *N. gonorrhoeae* in the throat, the swab specimen should be inoculated as soon as possible onto a selective medium, such as modified Thayer-Martin agar, to maximize yield. For diagnosis of diphtheria, both nasopharyngeal and throat swab specimens are collected and transported to the laboratory immediately. If laboratory personnel are not experienced in the recovery and identification of *C. diphtheriae*, the specimens should be sent in a semisolid transport media (e.g., Amies) to a reference laboratory. A differential inhibitory medium containing potassium tellurite, such as Tinsdale medium, is optimal for cultivating *C. diphtheriae*. However, this medium is expensive, has a short shelf-life, and is difficult to obtain from commercial vendors; therefore, it is seldom used in clinical laboratories. Colistin–nalidixic acid blood agar (CNA) is an acceptable alternative for the cultivation of *C. diphtheriae*, but because CNA is not a differential medium, all diphtheroid colony types must be evaluated to exclude *C. diphtheriae* when this agent is suspected. In addition, a sheep blood agar plate should be inoculated and examined for GAS.

Vincent's angina is an acute necrotizing ulcerative tonsillitis that may be caused by *F. necrophorum* and other anaerobes. A presumptive diagnosis may be made if gram-negative fusiform bacilli and spirochetes are seen in gram-stained smears prepared from a swab specimen of the ulcerated lesion. Cultures of the involved area are not usually helpful because many species of anaerobes are present in the oral cavity. However, blood cultures should be collected because the illness is commonly accompanied by sepsis.

SPUTUM AND TRACHEAL ASPIRATES

Microbiological studies of sputum (expectorated and induced) and tracheal aspirate specimens are done primarily to determine the etiologic agents of pneumonia and to assess mycobacterial infection. The Infectious Diseases Society of America and the American Thoracic Society have published guidelines for the diagnosis of community-acquired pneumonia (Metlay et al., 2019). Although routine sputum cultures are of questionable utility in many cases of community-acquired pneumonia, they should be performed if an unusual pathogen is suspected that would alter antimicrobial management. Clinical factors such as severe pneumonia requiring hospitalization or high risk for MRSA pneumonia, among others, support the utility of sputum culture (Metlay et al., 2019). Tracheal aspirates represent lower respiratory secretions collected in a Lukens trap from patients with tracheostomies. Patients with tracheostomies rapidly become colonized with gram-negative bacteria and other potential nosocomial pathogens and because bacteria colonizing the respiratory tract cannot be differentiated from bacteria causing invasive disease by culture of tracheal aspirates, interpretation of routine culture results is difficult. Culture for *Legionella* spp., mycobacteria, and fungi must be requested separately from routine culture because each of these requires special media for cultivation. *Pneumocystis jirovecii* cannot be cultured and is instead typically diagnosed using DFA staining; therefore, testing of *P. jirovecii* testing must also be requested separately (Miller et al., 2018).

Specimen Collection and Transport

Optimally, expectorated sputum is collected early in the morning before eating. The individual should rinse his or her mouth with water and then expectorate a specimen, preferably 5 to 10 mL, resulting from a deep cough. If mycobacteria are suspected, collection of three samples, at least one of which is an early morning specimen, is recommended. Additionally, the patient should not rinse his or her mouth before producing a specimen for mycobacterial workup because environmental mycobacteria that can be found in tap water such as *Mycobacterium gordonae* or *M. avium-intracellulare* could be introduced into the specimen. For persons with a nonproductive cough, a specimen may be induced by allowing the individual to breathe aerosolized droplets of a solution of 3% sodium chloride for about 20 minutes or until a cough reflex is initiated (Leber, 2016). Sputum and tracheal aspirate specimens should be collected into a sterile container and delivered promptly to the laboratory or refrigerated for a short time if a delay is unavoidable. If *Mycoplasma* is suspected, the specimen should be inoculated into liquid mycoplasmal growth media at the bedside if possible because the organism is very sensitive to environmental conditions such as drying (Leber, 2016).

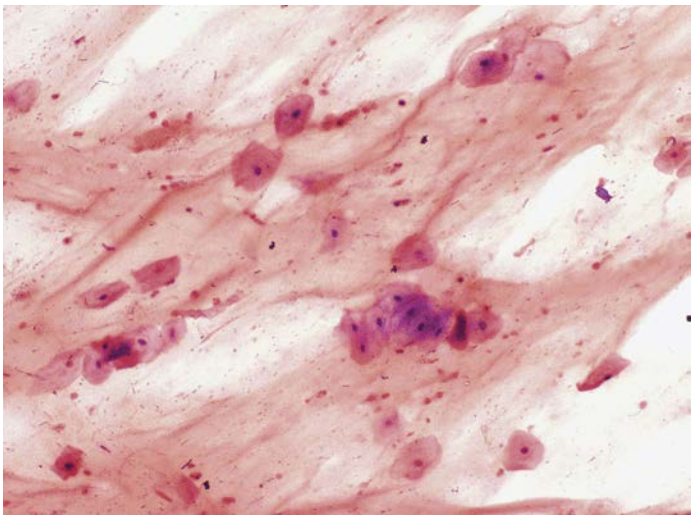

Figure 66.2 Gram stain of a sputum specimen demonstrating greater than 10 squamous epithelial cells per low-power field, which is unacceptable for culture. (10×.)

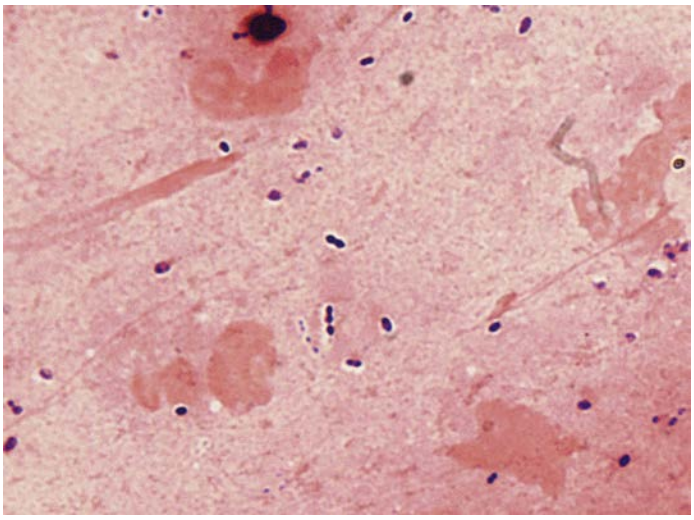

Figure 66.3 Gram stain of a sputum specimen showing encapsulated, lancet-shaped, gram-positive diplococci, consistent with *Streptococcus pneumoniae*. (100×.)

Specimen Processing

Sputum and tracheal aspirates for routine bacterial culture should be screened before they are plated to determine whether they are representative of lower respiratory secretions or of saliva. A smear prepared from a portion of the specimen consisting of purulent material is stained with the Gram stain. In general, specimens with more than 10 epithelial cells per low-power field (Fig. 66.2) are considered to have significant contamination with saliva and should be rejected. Specimens with fewer than 25 epithelial cells and more than 25 neutrophils per low-power field are probably acceptable (Murray & Washington, 1975). The number of neutrophils is not usually considered as a sole criterion when determining specimen quality because the individual from whom the sputum was collected may be neutropenic. Screening the quality of sputum samples submitted for detection of *M. pneumoniae*, *Legionella* spp., and mycobacteria is not generally required (Ingram & Plouffe, 1994; Havlik, 1995; McCarter & Robinson, 1996). The gram-stained smears prepared from specimens that are acceptable for culture are examined under oil immersion to determine the relative amounts of organisms. The quantity of organisms (rare, few, moderate, or many) is estimated for each kind of bacterium (e.g., gram-positive cocci in pairs [Fig. 66.3], chains, or clusters; gram-positive bacilli; gram-negative diplococci; and gram-negative rods), noting whether or not they are intracellular. Tracheal aspirates for which no organisms are observed in the gram-stained smear should probably be rejected (Morris et al., 1993). Portions of acceptable specimens containing purulent material are inoculated as outlined in Chapter 62. For specimens from persons with cystic fibrosis, also inoculating a medium selective for *Burkholderia cepacia* is recommended. The most sensitive method for detecting *B. pertussis* is PCR; a number of FDA-approved

assays are commercially available (van der Zee et al., 2015; US FDA, 2020). If PCR is not possible, *B. pertussis* culture should be performed as described above for nasopharyngeal samples.

When Legionnaires' disease is suspected, both *Legionella* culture and a rapid, direct test (e.g., fluorescent antibody on a respiratory specimen or *Legionella* antigen on a urine specimen) are recommended. DFA staining, which provides rapid results but is less sensitive than culture, can be used to supplement culture. Urinary antigen testing also provides rapid results but only detects *L. pneumophila* serotype 1 (Shimada et al., 2009). PCR can be performed but is considerably more expensive than culture, DFA, or urine antigen testing, and no FDA-approved assays are available yet (Benitez & Winchell, 2013). Culture, the most sensitive of these methods, can detect all *Legionella* spp. and serotypes and should always be performed. Decontaminating the specimens with acid (KCl-HCl buffer) before plating may enhance recovery of legionellae from sputum (Murdoch, 2003). Several drops of the specimen should then be inoculated onto each selective and nonselective buffered charcoal yeast extract agar plate. Use of the selective agar inhibits the growth of most other respiratory flora; however, some strains of *Legionella* spp. are susceptible to the medium's inhibitory agents. Thus, a nonselective plate should always be included.

For optimal detection of mycobacteria in sputum, the specimen must be decontaminated to prevent the normal respiratory flora from overgrowing the slower-growing mycobacteria. This process and detection methods are discussed in Chapter 61. All specimens submitted for mycobacterial workup should be processed in a biological safety cabinet, preferably in an isolated room with negative air pressure (Biosafety Level 3 laboratory).

All specimens submitted for fungal culture should also be handled as described for mycobacteria. The quality of the specimens should be determined by screening with smears stained with Gram stain (as described earlier for bacteria). Acceptable expectorated sputum, induced sputum, and tracheal aspirate specimens should be inoculated onto culture media for recovery of fungi. In general, to culture fungi, media with and without blood enrichment and media containing antimicrobial agents should be used. However, when making media selection, the laboratory director also should consider cost and the types of fungus usually encountered in the patient population served by the laboratory.

If viscous, sputum specimens for *P. jirovecii* detection by DFA staining are first treated with a mucolytic agent (e.g., N-acetyl-L-cysteine or dithiothreitol) and vigorously vortexed until the specimen is almost completely liquefied. After centrifugation, the sediment is smeared on glass slides and fixed, with fixation technique dependent on the stain manufacturer's instructions. Slides are then stained and examined microscopically (Leber, 2016).

BRONCHOSCOPY SPECIMENS

Bronchoalveolar lavage (BAL) fluid and protected brush specimens are useful for diagnosis of acute bacterial pneumonia, bacterial pneumonia in ventilated patients who have not received antimicrobial therapy and for detection of opportunistic pathogens in immunocompromised patients with pneumonia (Baselski & Wunderink, 1994; Carroll, 2002). BAL specimens may also be collected for suspected mycobacterial infection in patients who are unable to produce sputum (Holani et al., 2014). Although many have advocated quantitative cultures to improve the specificity of cultures from the lower respiratory tract, recent reviews have not shown an improvement in outcome for intubated patients compared with qualitative cultures (Caliendo et al., 2013). Only protected brush specimens are suitable for anaerobic culture (Baselski & Wunderink, 1994). For culture of *Legionella* spp., sputum specimens are preferable because BAL samples are diluted with saline and may contain small amounts of the anesthetic used locally, which inhibits the organism.

Specimen Collection and Transport

A protected brush sample is collected with a small brush that holds 0.001 to 0.01 mL of secretions, placed within a double-cannula enclosed catheter. The outer cannula has a displaceable polyethylene glycol plug at the tip. To obtain a specimen, the cannula is inserted to the desired area via bronchoscopy; the inner cannula is pushed out, dislodging the protective plug (water-soluble); and the brush is extended beyond the inner cannula. After the sample is taken, the brush is pulled back into the inner cannula, and both the brush and inner cannula are pulled into the outer cannula to prevent contamination of the brush when the catheter is removed. The brush is then placed into 1 mL of sterile saline or broth. The specimen should be transported immediately to the laboratory and processed as soon as possible. If a delay is unavoidable, the specimen should be stored in the refrigerator.

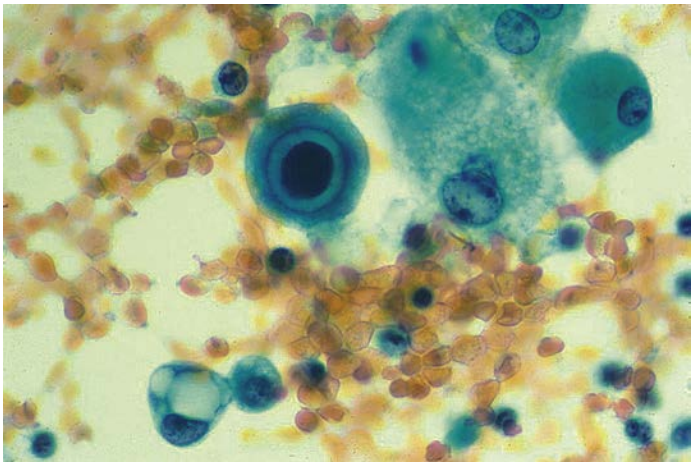

Figure 66.4 Cytologic preparation of bronchoalveolar lavage fluid shows an enlarged cell with intranuclear and intracytoplasmic inclusions, consistent with cytomegalovirus. (Papanicolaou stain, 250×.) (Courtesy of Vicki J. Schnadig, MD, Department of Pathology, University of Texas Medical Branch, Galveston, TX.)

To collect bronchoalveolar fluid, the tip of a bronchoscope is carefully wedged into an airway lumen. A volume of saline (usually >140 mL) in three to four aliquots is injected through the lumen, sampling an estimated 1 million alveoli. The total volume returned varies based on the volume instilled but is typically 10 to 100 mL. The specimen should be promptly transported to the laboratory (in <30 minutes) and processed as soon as possible because saline may be toxic to some respiratory pathogens (Leber, 2016). If a delay cannot be avoided, the fluid should be stored in the refrigerator. A number of FDA-approved NAATs for detection of respiratory viruses are available (US FDA, 2020). If a bronchoscopy specimen is being collected for testing by NAAT, the manufacturer's instructions for collection and processing should be followed.

Specimen Processing

To process the protected brush specimen, the fluid in which the brush is suspended is agitated on a vortex mixer, and the resulting suspension is used for a cytospin preparation and for culture inoculum. The BAL sample should be examined for small pieces of tissue; if present, they should be placed in a sterile container, kept moist with sterile saline, and processed in addition to the fluid (Sharp et al, 2004). If the specimen is viscous and fungi (including *P. jirovecii*) are suspected, treatment of the sample with a mucolytic agent (e.g., N-acetyl-L-cysteine or dithiothreitol) and subsequent centrifugation should be considered to concentrate contents and improve fungal recovery. The centrifuged specimen can then be used to prepare slides for staining (as discussed later) or inoculation to appropriate fungal culture media (outlined in Chapter 60).

Cytocentrifuge preparations can be useful for assessment of multiple types of respiratory pathogens. For bacterial detection, staining cytospin preparations with Gram stain is recommended because visualizing one or more bacteria per oil immersion field (in the absence of squamous epithelial cells) strongly suggests acute bacterial pneumonia (Kahn & Jones, 1987; Baselski & Wunderink, 1994). Examination of cytocentrifuge preparations stained with Papanicolaou stain allows detection of cytopathic changes, especially useful for diagnosis of CMV pneumonia (Fig. 66.4) (Woods et al., 1990). Cytospin preparations may also be stained with an acid-fast stain for detection of mycobacteria; with specific antibodies, such as those for detection of *Legionella* species or *P. jirovecii*; or with nonspecific stains (e.g., silver stain, Calcofluor white, or Giemsa) for detection of *P. jirovecii* or other fungi.

For bacterial culture, the laboratory may choose to inoculate plates quantitatively either by the serial dilution method or the calibrated loop method (Leber, 2016); however, the use of quantitative cultures is controversial, and pathologists should review their use with the clinical staff. The intent of this type of culture is to improve the specificity of the culture, with colony counts above a particular threshold appearing to correlate with infection.

For detection of mycobacteria, the specimen should be decontaminated and handled as described in Chapter 61. Viral detection methods from BAL specimens include NAAT and conventional cell culture, each further described in Chapter 64.

URINARY TRACT

The urinary tract above the urethra is sterile in healthy humans, but the urethra is normally colonized with many different bacteria, so urine specimens collected by a noninvasive method (e.g., clean-catch, midstream specimen) become contaminated during their passage. Growth of a predominant uropathogen in the quantity of 10^5 CFU/mL of bacteria or greater of urine has been considered highly indicative of infection. Quantitative cultures have been used to help differentiate between contaminants and presumed pathogens, but the entity of asymptomatic bacteriuria continues to confound culturing practices in the microbiology laboratory. Asymptomatic bacteriuria is the presence of bacteria in urine, with or without white cells, in patients (often women) who have no symptoms of urinary tract infection. This has led to the unnecessary use of antimicrobials. Some institutions have attempted to "screen" urine specimens, culturing only specimens meeting predetermined criteria often involving numbers of leukocytes or the presence of leukocyte esterase. Contemporary guidelines, bolstered by the *Choose Wisely* campaign to reduce inappropriate ordering of laboratory tests, instead focus on *not* culturing urine from nonpregnant, asymptomatic women (Nicolle, 2019). Growth of 10^5 CFU/mL of bacteria or greater of urine has been considered highly indicative of infection, but lower thresholds are relevant in certain populations. For example, in young, sexually active women with the acute urethral syndrome (dysuria, frequency, and urgency), as few as 10^2 CFU/mL is considered significant in the presence of concomitant pyuria (Stamm et al., 1982). True urinary infections associated with fewer than 10^5 CFU/mL may also occur in infants and children; in older men (Schaeffer & Nicolle, 2016); and in persons who are catheterized, were recently treated with antimicrobial agents, drink large amounts of fluids (which dilutes urine), have symptoms and concomitant pyuria, have urinary obstruction, or have pyelonephritis acquired from hematogenous spread (especially infections caused by yeast and *S. aureus*). Consequently, proper interpretation of urine culture results requires communication between clinicians and laboratory personnel.

SPECIMEN COLLECTION AND TRANSPORT

Acceptable methods of urine collection include midstream clean catch (preferably a first-voided morning specimen), catheterization, and suprapubic aspiration. In general, 24-hour urine specimens should be rejected except when detection of *Schistosoma haematobium* is requested specifically. Most commonly, the midstream flow of a clean-catch urine is collected. For women, the periurethral area and perineum should be cleaned before the specimen is collected (Nicolle, 2019). The first few milliliters of urine are passed into the toilet bowel or a bedpan to flush out bacteria normally colonizing the urethra, and the midstream portion is collected in a sterile container with a wide mouth and tightly fitting lid.

Catheterization is associated with the risk for inducing a nosocomial infection and should therefore be restricted to persons who are unable to produce a midstream sample, for example, individuals with an altered sensorium or those unable to void for neurologic or urologic reasons. Using strict aseptic technique, the catheter is inserted into the urethra, the first few milliliters of urine passed are discarded to clear organisms that may have entered the tip of the catheter during placement, and the midportion of the sample is obtained for culture. Urine may be collected from an indwelling catheter by aspirating with a 28-gauge needle and syringe through the rubber connector between the catheter and the collecting tubing, taking care to first disinfect the puncture site. Urine should not be collected from catheter bags, and Foley catheter tips should not be accepted for culture because they almost always are contaminated with urethral organisms.

Suprapubic aspiration is used primarily for neonates. The procedure requires a full bladder; the overlying skin is disinfected, the bladder is punctured above the symphysis pubis with a 22-gauge needle on a syringe, and about 10 mL of urine is aspirated.

All urine specimens should be transported promptly to the laboratory and should be processed within 2 hours after collection. If a delay in transport or processing cannot be avoided, specimens may be refrigerated up to 24 hours. Collection kits containing preservatives to maintain the bacterial population stable for 24 hours at room temperature are commercially available and advantageous if refrigeration cannot be guaranteed.

SPECIMEN PROCESSING

Quantitative bacterial culture of a urine specimen is done by inoculating appropriate media with a measured amount of urine, most commonly with a plastic or wire-calibrated loop designed to deliver a known volume.

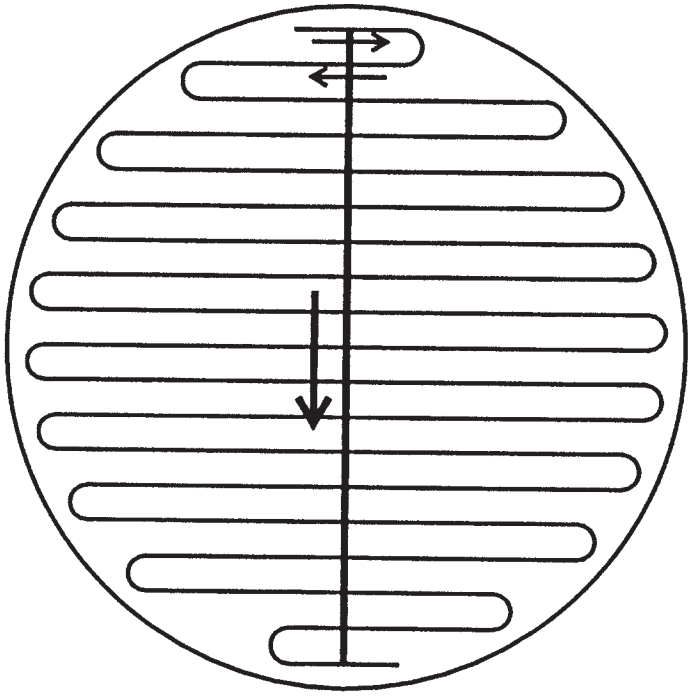

Figure 66.5 Technique for inoculating urine onto agar plates. (Redrawn from Woods GL, Gutierrez Y, editors: *Diagnostic pathology of infectious diseases,* Philadelphia, 1993, Lea & Febiger, p. 602.)

A 0.001-mL loop is used to inoculate all urine specimens except those collected from women with suspected acute urethral syndrome and suprapubic aspirates. Both the latter are inoculated with a 0.01-mL loop. The appropriate loop is inserted vertically into the well-mixed urine sample, and the loopful of urine removed is spread over the surface of the agar plate as illustrated in Figure 66.5. Without reflaming, the loop is again inserted vertically into the urine, and the removed sample is inoculated onto a second plate.

Some bacteria are not detected by routine culture of urine; when these pathogens are suspected, specific tests must be requested. For example, urine is an acceptable specimen for detection of *N. gonorrhoeae* and *C. trachomatis* by NAAT. The manufacturer's directions for urine collection and processing must be followed. *Leptospira interrogans* may be detected in urine after the first week of illness and for several months thereafter. To detect *L. interrogans* in urine, the specimen should be processed as soon as possible after collection because acidity may harm the organisms. One or 2 drops of undiluted urine and urine diluted 1:10 in broth are inoculated to 5 mL of Fletcher's medium or Ellinghausen-McCullough-Johnson-Harris medium containing 5-fluorouracil. Culture of urine for mycobacteria is discussed in Chapter 61. Yeasts may be recovered from urine on the media plated for routine bacterial culture, but if fungal culture is specifically requested, the sediment of a centrifuged urine specimen should be plated onto media such as inhibitory mold or SABHI agar containing antibacterial agents.

Urine specimens collected for culture of viruses should be submitted in liquid media containing antibiotics (e.g., penicillin, gentamicin, and amphotericin B), or antibiotics should be added to the sample when it is received in the laboratory to minimize bacterial contamination of cell cultures. PCR is more typically used than viral culture to identify viruses such as CMV, adenovirus, and HSV. Detection of BK virus in urine may be requested; however, PCR, rather than culture, is recommended. Because BK virus PCR is offered primarily by reference laboratories, it is best to contact that laboratory for collection and shipping requirements.

More than half of urine specimens submitted to the clinical laboratory for culture yield no growth or have bacterial counts below levels considered clinically significant. As discussed above, samples from asymptomatic, nonpregnant patients should not be cultured or screened for culture. Screening tests are used to quickly identify urine samples which are more likely to have "positive" culture results and to provide rapid results, eliminate negative specimens, and allow more time for positive specimens, improving efficiency and cost. In general, urine screen and culture results correlate well when 10^5 CFU/mL or greater is the reference, but they compare less favorably in the presence of lower colony counts. Commercial automated screening methods have been developed but are either no longer available or not actively marketed. Screening urine specimens by staining with

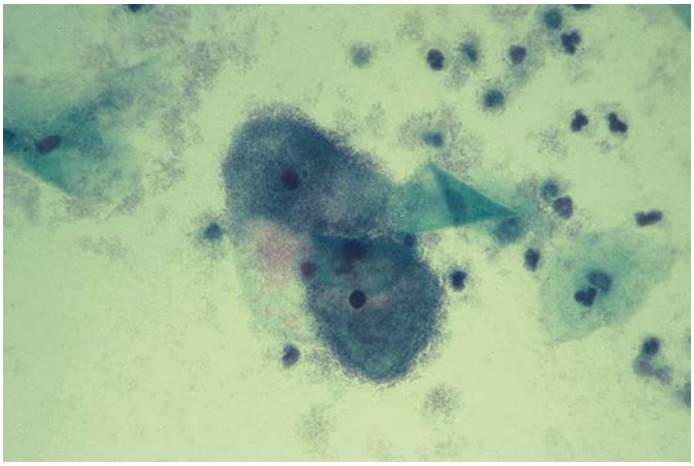

Figure 66.6 "Clue cells" in a smear of vaginal discharge. (Papanicolaou stain, 400×.) (Courtesy of Vicki J. Schnadig, MD, Department of Pathology, University of Texas Medical Branch, Galveston, TX.)

Gram stain is economical, but because examination of smears is tedious and time consuming, this approach rarely is adopted in the clinical laboratory. The commercial dipstick test that combines nitrate reductase (an enzyme present in most of the gram-negative bacilli that cause urinary tract infections) and leukocyte esterase (an enzyme produced by neutrophils) is rapid, inexpensive, and simple to perform and can suggest that a urinary tract infection is absent if both markers are negative, especially in symptomatic patients (Deville et al., 2004; St. John et al., 2006).

GENITAL TRACT

VAGINAL SECRETIONS

Vaginal secretions are useful in determining the etiologic agent of vulvovaginitis and bacterial vaginosis (BV). In women, the most common cause of BV is overgrowth of *Gardnerella vaginalis* (in association with a variety of anaerobes), whereas vulvovaginitis is most commonly caused by *Candida* spp. and *Trichomonas vaginalis*.

Regardless of the testing method, vaginal secretions should first be collected from the mucosal membrane of the vaginal wall using a sterile swab, with use of a manufacturer-provided swab if a testing kit is utilized.

Molecular assays are generally most sensitive for diagnosis of vulvovaginitis and BV. Three assays are currently available for simultaneous detection of *Candida* spp., *T. vaginalis*, and *G. vaginalis:* a DNA probe assay (Affirm VPIII microbial identification system, Becton Dickinson), a quantitative multiplex PCR assay (NuSwab, Laboratory Corporation of America Holdings), and an FDA-approved microbiome-based multiplex PCR assay (BD Max Vaginal Panel, Becton Dickinson) (Coleman & Gaydos, 2018). When testing by molecular methods, the manufacturer's protocol for specimen collection, transport, and processing should be followed.

If BV is suspected, a Gram stain of vaginal secretions should also be considered. When interpreted with a standardized scoring system (e.g., the Nugent score), the Gram stain is the most specific test for diagnosis of BV (Nugent et al., 1991). Culture is not recommended for diagnosis because *G. vaginalis* can be found in healthy women (Janulaitiene et al., 2017).

Although several rapid point-of-care tests exist for diagnosis of BV and vulvovaginitis (e.g., wet-mount preparations, vaginal pH test, whiff test), the sensitivity and specificity of these methods vary widely, and they are considered suboptimal (Miller et al., 2018). A wet mount is performed by placing a swab of the discharge in a tube containing about 1 mL of normal saline, removing the swab from the saline, and pressing the tip against a glass slide to express cellular material. The slide is coverslipped and examined for "clue cells" (epithelial cells covered with small coccobacillary bacteria) (Fig. 66.6), consistent with the diagnosis of nonspecific vaginosis; pseudohyphae, suggestive of candidiasis; and motile trichomonads. Other point-of-care tests include the vaginal pH test and the whiff test. The vaginal pH is usually about 4.5 in women with vulvovaginal candidiasis but is above 4.5 in those with bacterial vaginosis or trichomoniasis. A positive whiff test is the generation of a pungent, fishy odor after addition of 10% potassium hydroxide to 1 drop of vaginal discharge placed on a slide or on the speculum. This is associated predominantly with bacterial vaginosis but occasionally occurs with trichomoniasis.

ENDOCERVICAL AND URETHRAL SPECIMENS

Endocervical specimens are collected to determine the etiologic agents of cervicitis and to identify asymptomatic persons infected with an organism that cause sexually transmitted disease (e.g., *C. trachomatis*, *N. gonorrhoeae*). Endocervical specimens are obtained after the cervix is visualized with the aid of a speculum moistened only with warm water because lubricants may contain antibacterial agents. If a Papanicolaou smear is indicated, that sample should be collected first.

Specimens for microbiological studies are generally collected with a swab. Using a polyester-tipped swab with a plastic shaft is recommended. If a test kit is used for organism detection, the specimen must be collected with the swab specified by the manufacturer. Before collecting specimens for detection of *N. gonorrhoeae* and *C. trachomatis*, all discharge must be removed from the cervical os. The swab or brush then is inserted 1 to 2 cm into the endocervical canal (past the squamocolumnar junction), rotated firmly against the wall for 10 to 30 seconds, withdrawn without touching the surface of the vagina, and placed in the appropriate transport medium or tube system used to prepare a slide for DFA staining (for *C. trachomatis*) or used to immediately inoculate an agar medium for recovery of *N. gonorrhoeae*. Specimen handling varies based on the organism sought.

NAAT is recommended for detection of *N. gonorrhoeae* and *C. trachomatis*; several FDA-approved assays are available for simultaneous detection of both organisms (US FDA, 2020). If molecular methods are not available, culture may be performed, though sensitivity is lower (CDC, 2014). To isolate *N. gonorrhoeae*, direct inoculation of a selective agar medium, such as modified Thayer-Martin, within a container to which a CO_2-generating tablet is added, is optimal. Alternatively, the swab specimen can be placed in a tube transport system and delivered to the laboratory within 2 hours. If a delay in transport cannot be avoided, the swab should be left at room temperature and never refrigerated. Note that the viability of *N. gonorrhoeae* in Amies or modified Stuart medium substantially decreases after several hours (Miller et al., 2018). For chlamydial culture, the specimen should be transported in a transport medium containing antibiotics (e.g., M4 medium) to inhibit overgrowth of bacteria and fungi. To maintain the viability of *Chlamydia* organisms, the specimen should be transported to the laboratory and processed immediately. If delays in transport or processing are unavoidable, specimens should be stored in the refrigerator if they can be processed within 48 hours. Immunofluorescent staining (for chlamydial elementary bodies) and EIA are also available for chlamydial testing; the manufacturer's instructions for specimen handling must be followed.

To detect *C. trachomatis* and *N. gonorrhoeae* in men, a urethral swab specimen or first-voided urine sample, depending on the detection method used, should be obtained. Optimally, urethral swab specimens are collected at least 2 hours after the patient has voided. Samples for *N. gonorrhoeae* are obtained first, which maximizes the bacterial load and thus the likelihood of isolation (CDC, 2014). The conditions concerning types of swab and specimen transport are the same as those described for endocervical swab specimens, although the smaller urogenital swabs are used. The swab is inserted into the urethra for 2 to 4 cm, rotated in one direction for 5 seconds, withdrawn, and placed in the appropriate transport medium or used to prepare smears for DFA staining to detect *C. trachomatis* or for Gram staining to diagnose gonorrhea. Detection of intracellular gram-negative diplococci in a smear of urethral discharge from symptomatic men provides a presumptive diagnosis.

For optimal detection of HSV, NAAT is recommended, with specimen handling as per the test manufacturer or reference laboratory to which the sample is being sent. Alternatively, viral culture may be performed. The swab specimen is placed in viral transport medium such as M4 and transported as soon as possible to the laboratory. If immediate delivery is not feasible, the specimen should be stored in the refrigerator. For patients who have visible lesions on the cervix, a DFA may be performed on smears prepared from scrapings of the lesion base, though sensitivity is only about 70% compared with NAAT (Miller et al., 2018).

Genital tract infection with human papillomavirus (HPV) is diagnosed using nonculture methods; culturing the virus in vitro is not possible. Molecular techniques are most sensitive, with several available FDA-approved assays for testing of HPV genotypes associated with high risk for cervical cancer (US FDA, 2020). HPV-induced squamous cell changes may also be visualized on exfoliated cell samples (Papanicolaou smear).

VESICLES

Vesicular genital lesions are sampled to confirm HSV infection or (in children) atypical VZV infection. The vesicle fluid is aspirated with a small-gauge needle and syringe or capillary pipette. If only a small vesicle is

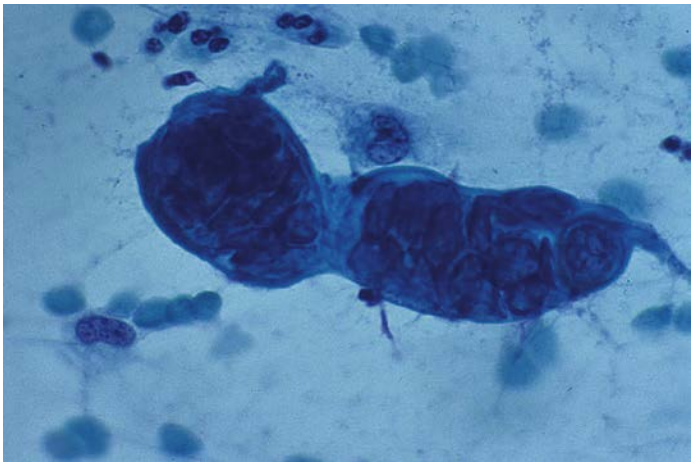

Figure 66.7 Multinucleate giant cell with intranuclear inclusions consistent with herpes simplex virus in a smear of endocervical cells. (Papanicolaou stain, 400×.) (Courtesy of Vicki J. Schnadig, MD, Department of Pathology, University of Texas Medical Branch, Galveston, TX.)

present, it is unroofed, and the base is firmly scraped with a Dacron swab to ensure collection of cells. Vesicle fluid or swab specimens should be placed in a viral transport medium. Testing by NAAT is optimal because it is most sensitive and can provide HSV typing information (Miller et al., 2018). HSV and VZV may also be detected in clinical specimens by viral cell culture or direct staining of smears, but these methods are less sensitive, and a negative result does not exclude the diagnosis. To make a smear, cells collected with a Dacron swab are spread on a glass slide, and the material is fixed in acetone for 5 to 10 minutes. The smear may be stained with Papanicolaou, Wright's, or Giemsa stain for detection of cytopathic changes (HSV and VZV have an identical appearance) (Fig. 66.7) or with specific monoclonal antibodies.

ULCERS

Material from genital ulcers is collected to identify the responsible pathogen; HSV, *Haemophilus ducreyi*, *Treponema pallidum*, *C. trachomatis* (serovars L1, L2, L3), and *Klebsiella granulomatis* should be considered.

For detection of HSV, specimen collection, transport and processing are identical to the protocol discussed for vesicles. In general, recovery of the virus and sensitivity of the tests previously described (i.e., NAATs, antigen detection, cell culture) are lower in ulcerative lesions compared to vesicular lesions.

Infection with *H. ducreyi* is typically diagnosed clinically (Workowski et al., 2015). If laboratory testing is deemed necessary, NAATs are generally most sensitive; several strategies have been developed for direct testing of clinical specimens, but there are currently no FDA-approved molecular assays for diagnosis of chancroid (West et al., 1995; Lewis, 2000; Chen & Ballard, 2012). Gram stain and culture are also possible but are less sensitive and not recommended unless the laboratory is experienced in these methods (Miller et al., 2018). For culture, a Dacron swab should be used to collect material from the base of the ulcer and directly inoculate a chocolate agar plate at the bedside. If bedside inoculation is not possible, the swab should be transported in modified Stuart's medium to the laboratory and held at room temperature until processed. A swab specimen may also be smeared onto a glass slide, Gram stained, and examined for small pleomorphic gram-negative coccobacilli in groups of chains resembling a "school of fish."

T. pallidum, the causative agent of syphilis, is usually diagnosed serologically. Spirochetes may be detected in genital or other lesions by darkfield microscopy or DFA, but these methods are unavailable in most laboratories. To collect specimens for spirochete detection, the surface of the lesion (if multiple lesions are present, the youngest should be selected) is cleaned with saline and blotted dry, and crusts are removed if present. The lesion is superficially abraded until slight bleeding occurs, and gentle pressure is applied to its base. The clear serum exudate from the subsurface is collected by directly touching the fluid with a glass slide or by aspiration with a 26-gauge needle (followed by drawing up 1 drop of saline into the syringe) with transfer of the fluid to a glass slide. A coverslip is placed on the fluid, and the specimen is examined immediately by darkfield microscopy for motile spirochetes or stained for DFA testing.

Lymphogranuloma venereum, caused by serovars L1, L2, or L3 of *C. trachomatis*, is typically diagnosed clinically. Serologic testing by microimmunofluorescence-IgG or complement fixation may be performed adjunctively (Stoner & Cohen, 2015). Although cell culture on biopsy or cellular material is possible, sensitivity is only 30%. Any overlying exudate is first removed from the lesion, and then a swab is firmly rotated against its base. Specimen transport and processing are the same as discussed for endocervical swab specimens.

K. granulomatis infection, which causes granuloma inguinale, is also typically diagnosed clinically. For laboratory examination, a scraping from an area of active granulation is taken, placed into formalin, and transported to the laboratory. The scrapings are then stained by Giemsa or Wright's stain for visualization of Donovan bodies, which appear as blue rods with prominent polar granules.

FECES

Feces and, in some cases, rectal swab specimens are useful for determining the etiologic agent of infectious diarrhea or food poisoning, confirming the diagnosis of diarrhea caused by *C. difficile* or *botulinum*, detecting certain sexually transmitted pathogens (e.g., *C. trachomatis*, *N. gonorrhoeae*), identifying carriers of bacterial pathogens for infection control purposes (e.g., vancomycin-resistant *Enterococcus* spp.), and in some instances, diagnosing helminths of the respiratory and biliary tracts. Collection, transport, and processing of specimens are different for viruses, bacteria, and parasites and are discussed separately for each group. Just as molecular and mass spectrometry methods have revolutionized the diagnosis of blood and respiratory specimens, they are similarly changing stool specimen diagnosis. There are numerous FDA-approved multiplex molecular methods for the rapid identification of bacterial, protozoal, and viral causes of diarrhea, as well as assays for identification of *C. difficile* and its toxin(s) (US FDA, 2020). Theoretically, this could eliminate routine stool cultures and ova and parasite examinations. However, as laboratories convert from culture-based to culture-independent methods, they must be aware of local regulations regarding submission of specimens or isolates of public health significance to public health laboratories and the need to perform susceptibility testing of certain pathogens (e.g., *Shigella* spp. and *Salmonella* spp. recovered from select age groups). Many jurisdictions ask that laboratories continue to send the patient's specimen for epidemiologic analysis.

VIRUSES

Specimen Collection and Transport

Stool is preferred for detection of enteroviruses and the viruses responsible for gastroenteritis (e.g., astrovirus, calicivirus, enteric adenovirus, rotavirus). Specimens should be collected in a clean container with a tight lid, or in VTM, depending on the type of testing to be performed. If feces cannot be obtained, a swab is inserted beyond the anal sphincter, rotated, withdrawn, and placed in VTM. Specimens should be delivered promptly to the laboratory; if this is not possible, fresh stool specimens can be stored at 4°C for up to 3 days and transported on wet ice. If the specimen must be mailed to a reference laboratory, it should be stored at –70°C and shipped on dry ice.

Specimen Processing

NAAT is recommended for detection of viral causes of gastroenteritis and enteroviruses because of their speed and accuracy of viral detection. A variety of laboratory-developed and commercial assays exist (US FDA, 2020). Viral culture remains an alternative method for adenovirus and enterovirus/parechovirus diagnosis, while EIA is an option for detection of rotavirus and adenovirus 40 and 41 (Miller et al., 2018). Specimens are processed according to the manufacturer's directions.

BACTERIAL PATHOGENS

Collection and Transport

The use of culture-independent diagnostic tests for bacterial stool pathogens is rapidly increasing because of their rapid turnaround time and improved sensitivity relative to culture (Shane et al., 2017). Many FDA-approved NAAT-based single- or multiplex assays are available (US FDA, 2020). Regardless of the testing method, stool is preferred for detection of pathogenic bacteria. Rectal swab specimens are an alternative but are less sensitive for culture in adults (Miller et al., 2018). Swabs should generally be reserved for detection of *N. gonorrhoeae*, *C. trachomatis*, and *Shigella* spp. and for assessment of vancomycin-resistant *Enterococcus* (VRE) carriage (Leber, 2016). Stool specimens should be collected in a clean container

with a tight lid, and the specimen should not be contaminated with urine, barium, or toilet paper because these substances can affect pathogen viability. Rectal swab specimens are obtained by inserting a swab past the anal sphincter (deep enough to become visibly stained with stool), gently rotated, withdrawn, and placed in a tube transport system containing appropriate transport media (e.g., Cary-Blair medium, manufacturer's transport medium for NAAT). All specimen types should be transported to the laboratory within 2 hours and quickly processed because the drop in pH that occurs as the stool cools may inhibit the growth of some pathogens, especially *Shigella* spp. If a delay in processing is unavoidable or if the specimen must be sent to a reference laboratory, transport of an aliquot of the specimen in transport media such as Cary-Blair is recommended.

Clinicians may consider testing a second stool specimen in adult patients who initially test negative for bacterial pathogens, but this is rarely necessary in pediatric patients. In adults, an enteric bacterial pathogen was detected in 87% of initial stool specimens, with a second specimen increasing this rate to 98% (Rohner et al., 1997). However, in pediatric patients, 98% of initial specimens were positive for a bacterial pathogen (Church et al., 1995).

Stool cultures for patients with diarrhea who have been hospitalized for more than 3 days are inappropriate unless the patient has been consuming food brought into the hospital. Offering *C. difficile* testing for health care–associated diarrhea may be considered instead. For diagnosis of *C. difficile* disease, 20 to 50 mL of liquid stool should be submitted in a sterile container. The stool specimen is stable for 2 days refrigerated or 1 week frozen. Testing should be performed only on patients with three or more unformed stools in 24 hours and who have not recently received laxatives. Unless the patient has ileus, testing of formed stool should be avoided because up to 15% of individuals are asymptomatic carriers, and testing may result in misdiagnosis and unnecessary treatment. Routine testing of children younger than 2 years old is not recommended because toxigenic *C. difficile* colonization is common in this age group (McDonald et al., 2018).

Specimen Processing

Processing stool or rectal swab specimens for detection of bacteria is based on the organism or group of organisms expected to be present. Specimens received for "routine" bacterial culture should be processed to allow recovery of *Shigella* spp., *Salmonella* spp., and *Campylobacter jejuni/coli* by plating to appropriate differential, inhibitory, and noninhibitory media. The microbiology laboratory director also might consider routinely examining stool for *Shiga* toxin–producing *E. coli* (STEC) and *Aeromonas* spp., depending on the prevalence of disease caused by these bacteria. The prevalence of gastroenteritis caused by *Yersinia enterocolitica*, *Vibrio cholerae*, or other *Vibrio* spp. or *Plesiomonas shigelloides* is low in most parts of the United States; therefore, specific requests for their detection are most cost effective. The patient population, season, and locale cause different laboratories to look for different pathogens. For this reason, every report from a stool culture should list the organisms that were identified or ruled out by the laboratory's protocol.

To detect STEC, *Shiga* toxin EIA or NAAT is preferred because they are more sensitive than culture and detect all serotypes of the organism (Pulz et al., 2003; Gavin et al., 2004). If culture is the only available method, the stool specimen is inoculated onto sorbitol-MacConkey agar (containing 1% D-sorbitol instead of lactose), a medium that differentiates isolates of STEC, which do not ferment sorbitol, from almost all other *E. coli*, which are sorbitol positive. When isolation of *Y. enterocolitica* is requested, cefsulodin-irgasan-novobiocin (CIN) agar is inoculated and incubated at room temperature. The organism also can be recovered by inoculating media typically used for routine bacterial culture. A MacConkey plate may be incubated at room temperature for 48 hours. Colonies of *Y. enterocolitica* are purple and pinhead in size. *Vibrio* spp. frequently grow on the media used for routine stool culture, but for their optimal recovery, thiosulfate citrate bile salts sucrose agar is inoculated. *Plesiomonas shigelloides* also grows on media used for routine culture, but because up to 30% of *P. shigelloides* isolates ferment lactose, their colonies do not appear sufficiently distinct to be recognized on these media, and screening all colonies for *Plesiomonas* is not cost effective. For this reason, culture of stool or rectal swab specimens for *P. shigelloides* should be requested specifically. Use of the selective-differential medium inositol brilliant-green bile salts agar has been suggested but is not essential.

Rectal swab specimens submitted for detection of *C. trachomatis* and *N. gonorrhoeae* are placed in transport medium and delivered promptly to the laboratory or are refrigerated for a short time. NAATs are recommended for detection of these organisms because they have superior sensitivity to culture and are highly specific (Schachter et al., 2008). Although NAATs are not FDA approved for extragenital site testing, they can be validated for this purpose.

Stool specimens or gastric contents collected from persons with short-incubation food poisoning should be evaluated for toxins from *S. aureus* and *Bacillus cereus*, and specimens from patients with long-incubation food poisoning should be evaluated for *Clostridium perfringens*. This testing is normally conducted by experts at public health laboratories, who should be contacted regarding appropriate specimen collection and transport. The clinical diagnoses of foodborne botulism and infant botulism may be confirmed by detecting botulinal toxin, *Clostridium botulinum*, or both in feces. Most clinical laboratories are not properly equipped to process specimens from persons with suspected botulism. In the United States, when a case of botulism is identified, investigators at the CDC should be notified to ensure appropriate specimen collection and transport, diagnosis, treatment, and investigation of the potential outbreak.

Diseases associated with *C. difficile* infection (CDI), such as pseudomembranous colitis and antibiotic-associated diarrhea, are caused by the toxins produced by the organism. In conjunction with clinical symptoms, several laboratory tests are available as diagnostic aids for CDI. These include toxigenic culture (TC); the cell cytotoxicity neutralization assay (CCNA); EIAs and immunochromatographic assays for detection of glutamate dehydrogenase (GDH) antigen, toxin A or B, or both toxins; and NAATs. TC and the CCNA serve as two reference methods for *C. difficile* diagnosis; however, both are slow and labor intensive. A testing algorithm that uses NAAT alone or in combination with GDH or toxin detection by immunoassay is recommended as an alternative approach for *C. difficile* detection (Kraft et al., 2019). NAAT-only testing has been shown to be highly sensitive and specific for detection of the *C. difficile* toxin genes A and B (Kraft et al., 2019). However, a NAAT-only approach may be cost prohibitive for laboratories, especially when testing volumes are high. Although immunoassays for GDH have low specificity for CDI because they do not distinguish isolates that produce toxin from those that do not, they have high sensitivity and a rapid turnaround time (15–45 minutes). Therefore, some have adopted a multistep algorithm by which samples are first screened with a GDH assay. Specimens yielding negative results require no further testing, whereas GDH-positive specimens are tested for toxin(s) using EIA or NAAT (Leber, 2016).

For epidemiologic studies, *C. difficile* may be isolated from stool or from rectal swab specimens placed in an anaerobic transport system. Because many bacteria are present in stool, procedures that select for *C. difficile* must be used. The most effective medium for this purpose is cycloserine cefoxitin fructose egg yolk agar (CCFA), with or without horse blood. The organism grows more quickly and luxuriantly on formulations containing horse blood. The medium is incubated anaerobically for 48 hours, and the plates are examined for colonies that have a peripheral fringe and a "horse stable" odor. Alternatively, *C. difficile* may be isolated by using an alcohol spore selection procedure, in which 1 mL of the original specimen is mixed with 1 mL of absolute ethanol. The mixture is allowed to stand for 1 hour at room temperature followed by subculture to CCFA and incubation anaerobically.

Swabs for assessment of VRE carriage can be inoculated to selective media containing vancomycin or VRE chromogenic agar, with subsequent NAAT-based testing to differentiate *vanA*- versus *vanB*-containing isolates (Leber, 2016).

With regard to mycobacterial culture, stool specimens usually are submitted for isolation of *Mycobacterium avium* complex (primarily from patients with AIDS), but *M. tuberculosis* complex and other *Mycobacterium* spp. also may be recovered. Processing the specimen (1–2 g of formed stool or 5 mL of liquid stool) involves decontamination and concentration, preparation of smears, and inoculation of media as discussed in Chapter 58.

PARASITES

The backbone of diagnostic parasitology in clinical laboratories is examination of stool samples for parasitic protozoa and helminth eggs or larvae. Laboratories performing such tests should have adequate facilities for handling stool samples and a good microscope with a calibrated micrometer to measure the organisms found. Staining of fecal smears is also required for identification of intestinal protozoa. In addition to morphologic examination, several antigen- or NAAT-based commercial assays have been developed for diagnosis of intestinal parasites (e.g., *Giardia* spp., *Cryptosporidium* spp.) as part of a gastrointestinal pathogen panel (US FDA, 2020).

Specimen Collection and Transport

The specimen (usually collected by the patient) can be collected in a clean, dry, wide-mouthed container. A portion of the sample is then aliquoted into two vials containing preservatives, one with modified polyvinyl alcohol (PVA) and the other with 10% formalin. Fecal specimens should not be

collected from the toilet bowl and should not be contaminated with urine, water, mineral or castor oil, antidiarrheal compounds, or radiologic contrast medium because these can destroy organisms or interfere with their detection (Garcia, 2016). After being collected and preserved, the sample should be delivered to the laboratory at the patient's convenience. The use of fresh stool is optimal for examination for motile trophozoites; however, it is usually very difficult to transport a fresh specimen to the laboratory and into the hands of the microbiologist within the 30 to 60 minutes after collection required to visualize motile trophozoites. Although it is rarely possible to perform wet mounts on fresh stools for motile trophozoites, they can still be identified on stained preparations, and the use of preserved specimens for examination minimizes the exposure of laboratory personnel to live organisms.

Several kits are commercially available for collection and transport of fecal samples; the choice depends on testing to be performed (e.g., special stains, immunoassays, NAAT) and the test manufacturer's recommendations for specimen fixative (Miller et al., 2018). The question of how many fecal specimens are required for identification of all individuals with intestinal protozoa or helminths is still without a definitive answer. It has been advised that a minimum of three specimens be examined, with one specimen collected every other day (Garcia et al., 2003; Clinical and Laboratory Standards Institute, 2005). For cost containment, the clinician should request examination of only one specimen because about 90% of all infections are diagnosed in the first sample (Montessori & Bischoff, 1987). If a parasite is not detected in the first sample, a second or third should be requested. If two or more specimens collected on different days are received in the laboratory at the same time, it has been suggested they be pooled and evaluated as one (Peters et al., 1988). This practice, however, is controversial. Stool examinations for parasites in patients who have been hospitalized for more than 3 days are inappropriate unless they are immunocompromised (Siegel et al., 1990).

Specimen Processing

The examination of stool samples for parasites includes preparation of saline solution and of iodine (Lugol's)-stained wet mounts of freshly collected samples (i.e., those that can be examined within a few hours of collection). This is followed by concentration of cysts and helminth eggs and finally by preparation of smears for staining with the trichrome stain. The wet preparations, if performed, should be made and examined before doing the concentration and trichrome staining. Even if a parasite is seen in the wet mount, examination of a concentrated specimen is recommended because specimens submitted for ova and parasite examination may contain multiple parasites. In particular, helminth eggs are often only visible in the concentrate.

The standard fecal smear is made from a fresh fecal sample as follows: 1 drop of saline solution is placed on a clear glass slide. With an applicator stick, a small amount of feces is picked up and with circular movements is mixed thoroughly with the saline (until enough sample is dissolved), and the mixture is covered with a 22-mm square cover glass. A good wet smear prepared as described, if placed on a paper with small print, should allow the print to be read through the smear. The smear stained with iodine is prepared in the same manner. Both the saline solution and the iodine solution wet preparations can be made on the same glass slide, at the same time mixing the feces with the saline solution first and then with the iodine solution. The saline solution smear will show trophozoites and cysts of protozoa, plus all the helminth eggs and larvae. In addition, the saline solution shows movement of trophozoites, which is useful for their identification. The smear stained with iodine does not show trophozoites because they are destroyed by the iodine unless the sample was previously fixed. The main advantage of the iodine stain is that it allows better visualization of some morphologic characteristics of cysts. To prepare wet mounts from formalin-fixed material, the contents are mixed well, and 1 drop is placed directly onto the glass slide and onto 1 drop of iodine solution.

Examination of the saline solution smear is carried out with medium power (10× objective) at first. Beginning at the upper-left corner of the cover slide, the slide is moved horizontally from right to left. The operation is repeated until the entire 22-mm square cover glass is examined. All helminth eggs or larvae and all protozoal cysts should be noted. Examination with the high-power objective is done next for detection and identification of protozoa, looking randomly for about 5 to 10 minutes per preparation. Note that pathogenic *E. histolytica* and nonpathogenic *Entamoeba dispar* cannot be distinguished based on morphology alone; an immunoassay or NAAT is needed for species-level identification (Tanyuksel & Petri, 2003).

The concentration technique, which can be done both on fresh and fixed specimens, is particularly useful because it allows detection of organisms present in low numbers. The concentration methods used for routine evaluation are described in detail in Chapter 60. Also discussed in that chapter are special stains required for detection of *Cryptosporidium* spp., microsporidia, and *Cyclospora* spp.

SKIN AND SUBCUTANEOUS LESIONS

FLUID-FILLED LESIONS (VESICLES, PUSTULES, BULLAE, AND ABSCESSES)

Fluid-containing lesions can be from viral, bacterial, or fungal causes; the appearance and distribution of the lesions suggest the most likely cause and should guide the specimen collection strategy. Vesicles are often viral, with HSV and VZV being common culprits. However, a bacterial cause should be considered if vesicles are limited to the face or extremities, as in nonbullous impetigo. Pustules are usually bacterial. Bullae that are generalized over the body are also typically associated with bacteria, especially *Staphylococcus* spp., whereas bullae limited to the foot are usually fungal. Finally, abscesses can be either bacterial or fungal, with anaerobic bacteria especially likely in the case of a closed abscess.

Specimen Collection and Transport

Regardless of the cause, aspirated fluid is the optimal specimen for testing fluid-filled lesions because this maximizes the concentration of viable pathogen while limiting contamination by extraneous microbes. Before fluid collection for bacterial or fungal testing, the overlying skin should be disinfected and allowed to dry completely. However, if viral testing is desired, skin disinfection should be avoided because antiseptic agents may inactivate viruses (Williamson et al., 2017).

Fluid can be aspirated using a needle and syringe. If viral testing is to be performed, the fluid should be immediately rinsed into VTM. Otherwise, the needle can be removed from the syringe and the syringe capped for transport to the laboratory.

If fluid aspiration is not possible, swabs may be used for sampling. Flocked swabs are preferable to nonflocked swabs because they yield greater organism recovery (Van Horn et al., 2008). Any overlying crust should first be gently removed and the lesion unroofed if necessary. One swab should then be used to absorb any fluid present, and a second swab should be used to vigorously rub the base and margins of the lesion. A minimum of two swab specimens should be collected: one for culture and the other for preparation of smears for staining. However, if more than one group of organisms (e.g., bacteria and fungi, fungi and viruses) is to be tested, collecting *at least* three swab specimens is recommended.

Swabs for bacterial or fungal testing may be placed into a commercially available swab transport system, with use of an anaerobic transport system if anaerobes are being considered. If viral causes are suspected, at least one swab should be placed into VTM. In addition, viral immunofluorescent staining (e.g., for HSV and VZV) may be helpful; slides should be prepared at the bedside by rolling the entire surface of the swab over a glass slide and allowing the material to air dry. All specimens should be promptly transported to the laboratory to limit loss of fastidious organisms and overgrowth of contaminants.

Specimen Processing

Processing specimens for detection of bacteria or fungi involves preparation of a smear for staining with Gram stain (for bacteria) or Calcofluor white (for fungi) and inoculation of appropriate media for culture. Additionally, if fungi are suspected, the fluid should be examined for grains or granules with a dissecting microscope because their presence indicates a mycetoma (Walsh et al., 2018). If present, grains and granules should be teased out of the specimen, washed in sterile solution, and examined for color (Leber, 2016). One portion should be crushed between two glass slides for examination for hyphae, and a second portion should be crushed and inoculated directly onto appropriate media.

Methods for viral detection include NAATs, antigen detection, and cell culture, any of which can be used to test fluid or swabs in VTM. Swab specimens received in VTM should be vigorously agitated on a vortex mixer, with removal and disposal of the swab. Specimens can be briefly refrigerated until they are processed.

WOUND INFECTIONS

Specimen Collection and Transport

Optimal wound sampling methods include aspiration (collected and transported as discussed earlier for fluid-filled lesions), tissue biopsy, and curettage. Swabs are far less helpful because they often are contaminated by skin commensal bacteria, leading to diagnostic confusion (Lipsky et al., 2012;

Nelson et al., 2018). All specimens should be delivered promptly to the laboratory. If transport will be delayed, inject a portion of the aspirate into an anaerobic transport tube. Tissue samples are transported in a sterile cup with moist sterile gauze to prevent desiccation. If a delay is unavoidable, specimens may be stored in the refrigerator, except those for anaerobe recovery, which should be maintained at room temperature.

Specimen Processing

Specimens should be Gram stained and inoculated to appropriate media for culture, as discussed in Chapters 59 and 62.

ULCERS

Ulcers may be primarily infected (e.g., those caused by viruses, *Bacillus anthracis*, *C. diphtheriae*, *Francisella tularensis*, *P. aeruginosa*, mycobacteria, or fungi) or secondarily colonized with aerobic and anaerobic bacteria, as is typical for chronic ulcers.

Specimens for viral detection from cutaneous ulcers are collected and transported identically to those described above for fluid-filled lesions. For bacterial, mycobacterial, or fungal isolation, handling of specimens differs by suspected cause.

B. anthracis is the cause of cutaneous anthrax, a rare disease in the United States that occurs among persons working with raw wool and other animal products contaminated with spores. For optimal diagnosis, specimens should be sent to a public health laboratory where NAAT can be performed. The public health laboratory's instructions for specimen collection and transport should be followed. If specimens are processed in the clinical laboratory, they must be handled in a biological safety cabinet. The swab is inoculated onto sheep blood agar and incubated in ambient air.

C. diphtheriae causes cutaneous diphtheria, an ulcerative lesion covered with a layer of necrotic debris resembling a membrane. For optimal diagnosis, a smear for staining with methylene blue is prepared from material from the edge of the membrane, and two swab specimens from the membrane itself are collected. One swab is used for routine bacterial culture and the other for inoculation of media selective for *C. diphtheriae* (e.g., cysteine tellurite agar) if available.

Ecthyma gangrenosum is an ulcerative cutaneous lesion that almost always occurs during bacteremia with *P. aeruginosa*. Ideally, two swab specimens are collected from the ulcer base; one is used to prepare a smear for Gram staining and the other for culture.

The diagnosis of the ulceroglandular form of tularemia, caused by *F. tularensis*, requires collection of a swab specimen from the ulcer base. The swab should be processed in a biological safety cabinet for routine bacterial culture, and the plates should be held for seven days because *F. tularensis* is a slowly growing bacterium that produces pinpoint colonies in 3 to 5 days on chocolate agar but does not grow on sheep blood agar. Suspicious isolates should be referred immediately to the nearest public health laboratory.

Mycobacteria most commonly isolated from cutaneous ulcers include *Mycobacterium fortuitum*, *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobacterium marinum*, and *Mycobacterium haemophilum*. *Mycobacterium ulcerans* is a common cause of skin lesions in some parts of the world but is difficult to culture. Exudate aspirated with a needle and syringe or a tissue sample is optimal for recovery of mycobacteria. Swab samples are not acceptable because mycobacteria become entrapped in the fibers of the swab and are difficult to dislodge. Transport of specimens is identical to that described earlier for wound infections. Specimens may be refrigerated for a short time until they are processed. Because some species of mycobacteria infecting the skin and extremities grow better at lower temperatures or with additional growth factors, it is important that personnel processing specimens are aware of the specimen source.

For fungal detection, an aspirate of the exudate (handled as described earlier for aspirates of fluid-filled lesions) or tissue sample from the active margin of the ulcer is optimal. A swab specimen of the exudate is suboptimal. Processing the specimen for detection of fungi involves preparing a smear for direct microscopic examination (potassium hydroxide or Calcofluor white preparation) and inoculation of appropriate media for culture.

Chronic skin ulcers are often colonized by aerobic, facultative, or anaerobic bacteria. To identify the responsible organisms, cultures of deep tissue biopsy (after thorough debridement) or deep aspiration of purulent material by needle and syringe are most useful. Surface cultures of such ulcers are not valuable because they usually represent colonizing bacteria rather than the causative agent (Miller et al., 2018). Transport and processing of tissue and aspirated material are identical to those described earlier for wound infections.

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