

Laboratory Diagnosis of Infections Due to Blood and Tissue Parasites

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Microscopy remains the cornerstone of the laboratory diagnosis of infections due to blood and tissue parasites. Examination of thick and thin peripheral blood smears stained with Giemsa or other appropriate stains is used for detection and identification of species of *Plasmodium*, *Babesia*, *Trypanosoma*, *Brugia*, *Mansonella*, and *Wuchereria*. Even in the hands of well-trained technologists, diagnosis may be hampered by the sparseness of organisms on the slide and by the subjective nature of differentiating similar-appearing organisms. Microscopy and/or culture of ulcer, bone marrow, tissue aspirate, and biopsy samples are useful for the diagnosis of African trypanosomiasis, onchocerciasis, trichinosis, and leishmaniasis. Serologic assays are available for the diagnosis of a number of these infections, but none of these assays are sensitive or specific enough to be used on their own to establish a diagnosis. In particular, the use of assays for the diagnosis of infection with a particular helminth will often cross-react with antibodies to a different helminth. Very sensitive polymerase chain reaction assays have been developed for a number of these parasites and are available from the Centers for Disease Control and Prevention and from several referral laboratories.

Microscopy remains the cornerstone of diagnostic laboratory testing for blood and tissue parasites (table 1). The microscopic examination of thick and thin peripheral blood smears stained with Giemsa or other appropriate stains (see "Babesiosis and Malaria" section) is used for detection and identification of *Plasmodium*, *Babesia*, and *Trypanosoma* species and of the filarial nematodes species (ie, *Brugia*, *Mansonella*, and *Wuchereria*) [1]. Although they require a minimal amount of reagents and equipment, these methods of detection and identification depend for their accuracy on well-trained and experienced technologists. Diagnosis may be hampered by the sparseness of organisms on the slide and by the subjective nature of differentiating similar-appearing organisms. The clinician can aid the technologist in these examinations by providing any clinical and/or epidemiologic information that will help ensure appropriate testing and guide the interpretation of results. The sensitivity of these methods may be enhanced by concentration procedures (eg, buffy coat test, centrifugation, and filtration). Microscopic examination and/or

culture of ulcer, bone marrow, tissue aspirate, and biopsy samples are useful for the diagnosis of African trypanosomiasis, onchocerciasis, trichinosis, and leishmaniasis [2]. For all of these procedures, the samples must be properly obtained, they must be transported to the laboratory as quickly as possible, and they must be processed in a timely fashion to preserve organism viability and/or morphology.

Serologic assays for detection of antibodies are available as adjunctive methods for the diagnosis of a number of these infections. None of these assays are sensitive or specific enough to be used on their own to establish the diagnosis. In particular, the use of assays for the diagnosis of infection with a particular helminth will often cross-react with antibodies to a different helminth. An indirect fluorescent antibody assay can provide quantitative titer results, but reading the slides is subjective and inherently prone to variability. An enzyme-linked immunosorbent assay (ELISA) provides only qualitative positive or negative results that are determined by an arbitrarily set breakpoint. Thus, clinicians will not be able to determine whether a positive test result was either a very strong or a very weak positive test result, without calling the laboratory for more information.

A simple rapid immunochromatographic (ICT) card assay (BinaxNOW Malaria; Binax) for the detection of antigens of *Plasmodium* in blood has been approved by the US Food and Drug Administration (FDA) and is commercially available [3].

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Table 1. Overview of the Approach to the Diagnosis of Blood and Tissue Parasitic Infections

Disease and organism(s)	Diagnostic test(s)	Remarks
Babesiosis due to species of <i>Babesia</i>	Microscopy of thick and thin blood smears stained with Giemsa or other appropriate blood stains	Real-time PCR available from CDC and referral laboratories. Serology does not distinguish acute from past infection.
Lymphatic filariasis due to species of <i>Wuchereria</i> , <i>Brugia</i> , and <i>Mansonella</i>	Microscopy of thick and thin blood films stained with Giemsa or other appropriate blood stains. Microscopy of concentrated/filtered blood or buffy coat. Serology or antigen detection for <i>Wuchereria bancrofti</i> (CDC or referral laboratory).	Blood films may be best examined during night, depending on periodicity of microfilaria. Serology does not differentiate genera and species of filaria. Motile adult worms may be visualized by ultrasound exam of involved lymphatics.
Onchocerciasis due to <i>Onchocerca volvulus</i>	Microscopy of skin snip or skin biopsy	Skin snips should be from areas nearby nodules and should be "razor thin."
Cutaneous leishmaniasis due to <i>Leishmania major</i> and other species	Microscopic exam of Giemsa-stained biopsies or scrapings of base or leading edge of ulcers; culture may be available.	Histopathologic microscopy of ulcer base or leading edge biopsies may be diagnostic if scrapings are negative.
Visceral leishmaniasis due to <i>Leishmania donovani</i> and other species	Histopathologic microscopy of Giemsa-stained bone marrow; culture may be available. Serology using rK39 antigen available from CDC or referral laboratories.	Positive rK39 serology reported to indicate active infection
Malaria due <i>Plasmodium falciparum</i> , <i>P. ovale</i> , <i>P. vivax</i> , and <i>P. malariae</i>	Microscopy of thick and thin blood smears stained with Giemsa or other appropriate stains; antigen-detecting ICT strip tests	Antigen strip tests lack sensitivity when level of parasitemia is low, and they do not differentiate all 4 species of <i>Plasmodium</i> . Real-time PCR available from CDC and referral laboratories and will differentiate species.
Trichinosis, cysticercosis, and echinococcosis	Serology from CDC or referral laboratories	Encysted larvae and/or hooklets can be seen in histopathologic sections of tissue biopsies or aspirate samples of cysts.
African trypanosomiasis, or sleeping sickness, due to <i>Trypanosoma brucei gambiense</i> (West African) or <i>T. b. rhodesiense</i> (East African)	Microscopy of thick and thin blood smears or buffy coat preparations stained with Giemsa or other appropriate stains. CSF and aspirate samples of chancres and lymph nodes may also be examined.	A card agglutination test is available for serology of <i>T. b. gambiense</i> infection. Contact CDC or the Parasite Diagnostic Unit, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium (e-mail: info@itg.be; Web site: http://www.itg.be/itg/).
American trypanosomiasis (Chagas disease) due to <i>Trypanosoma cruzi</i>	Microscopy of thick and thin blood smears or buffy coat preparations stained with Giemsa or other appropriate stains. Serology available for donor and diagnostic testing. Culture of blood may be available using special media (eg, Novy-MacNeal-Nicolle agar).	IgG antibody may persist for decades, and its presence is considered evidence of chronic infection.

NOTE. Referral laboratories are laboratories that perform esoteric testing not usually done in routine hospital laboratories (eg, ARUP Laboratories [800-522-2787], Focus Diagnostics [703-480-2500], and Mayo Medical Laboratories [800-533-1710]). CDC, Centers for Disease Control and Prevention (specifically, the Division of Parasitic Diseases in Atlanta, GA [telephone, 770-488-4431; central telephone, 404-639-3311; available at: <http://www.cdc.gov/> or <http://www.dpd.cdc.gov/dpdx/>); CSF, cerebrospinal fluid; ICT, immunochromatographic; PCR, polymerase chain reaction.

Healthcare workers in emergency departments or clinics may use this assay to quickly establish a diagnosis of malaria while awaiting results of blood smears. This assay is adequately sensitive to use for symptomatic patients with malaria, but it loses its sensitivity if the level of parasitemia is very low. The Centers for Disease Control and Prevention (CDC) and a number of referral laboratories in the United States and Canada perform extremely sensitive real-time polymerase chain reaction (PCR) assays for some of these agents, including *Plasmodium* and *Babesia* [4–8].

BABESIOSIS AND MALARIA

The standard method for diagnosis of babesiosis or malaria is microscopy of Giemsa-stained thick and thin blood films. A Wright stain, a Wright-Giemsa stain, or a rapid stain (such as a Field stain) may also be used as long as the white blood cells (which stain the same as parasites) are adequately stained. Although this method requires a minimum amount of resources (staining materials and high-quality microscopes), well-trained and experienced technologists must be available. Blood film examinations for *Plasmodium* should be considered stat procedures, and ideally the slides should be examined within 2–3 h of obtaining blood samples from the patient. In addition, if blood is collected in tubes containing ethylenediaminetetraacetic acid, parasite morphology may be altered by delays in preparation of the slides. Thick blood films are essentially lysed concentrates that allow rapid detection of the presence of parasites consistent with either *Plasmodium* or *Babesia*, but these thick blood films generally do not allow for the definitive identification of a particular species of *Plasmodium* [1]. Careful examination of the thin film is used to identify whether a particular parasite and/or species is present and to determine the level of parasitemia. Recently, human infections with the simian parasite *Plasmodium knowlesi* have been described by Putaporntip et al. [9]. This organism has morphologic features similar to those of *Plasmodium malariae*, but it may cause severe clinical infections. Because it is difficult to specifically identify on blood smears, molecular testing may be very useful [9]. It is important to remember that *Babesia* and *Plasmodium* species may at times be indistinguishable on smears and that both can be transmitted by transfusion, so each can occur in atypical clinical settings. The quantitative buffy coat method detects fluorescently stained parasites within red blood cells and requires specialized equipment [6]. In addition, it requires preparation of a thin blood smear if a quantitative buffy coat sample is positive for parasites, because the specific identification and level of parasitemia will still need to be determined. Serologic testing is not the best method for diagnosing acute babesiosis, because antibodies may not appear during the early stage of infection; the use of smears and/or molecular testing is preferred. Serologic testing for malaria is not useful for patient diagnosis except perhaps for the rare tropical

splenomegaly syndrome. Malaria serology may be available by contacting the CDC.

Rapid real-time PCR assays for the diagnosis of babesiosis and malaria have recently been developed [7, 8] and are available from commercial referral laboratories (eg, Mayo Medical Laboratories in Rochester, Minnesota, and Focus Diagnostics, in Cypress California) and from the CDC. The methods are comparable in sensitivity to the methods used for thick blood film microscopy. PCR may be useful for accurate diagnosis of acute infection, if smears are negative or difficult to interpret, and for the differentiation of malaria parasites from *Babesia* or nonparasitic artifacts. Use of PCR may also confirm the diagnosis by detection of remnant nucleic acid in cases empirically treated without prior laboratory diagnosis. Similarly, PCR should not be used to monitor response to therapy, because the assay may remain positive for 7–10 days after intact parasites have been eradicated. The PCR for *Babesia* has been used as part of a “tick panel” of molecular testing in parts of the country where tick-transmitted infections frequently occur and the clinical syndromes are similar (babesiosis, ehrlichiosis, anaplasmosis, and Lyme borreliosis).

The BinaxNow rapid diagnostic test (RDT) for diagnosis of malaria has recently been approved by the FDA [3]. It is a rapid ICT card (or “dipstick”) assay that uses monoclonal antibodies to detect the histidine-rich protein 2 antigen of *Plasmodium falciparum* and an aldolase common to all species of *Plasmodium*. Positive RDT results should be confirmed by blood smears, which are also necessary to determine which species of *Plasmodium* other than *P. falciparum* (if the assay is positive for aldolase but negative for histidine-rich protein 2) is present and to determine the level of parasitemia. It may also be used to help differentiate malaria from babesiosis. This RDT is somewhat less sensitive than a thick blood film examination and may be falsely negative in cases with very low levels of parasitemia [10]. However, its sensitivity is comparable to that of examination of blood smears obtained from symptomatic malaria patients in endemic areas [11]. This may not be true for nonimmune patients who may be symptomatic with very low levels of parasitemia. In addition, RDT results may be falsely positive for several days after eradication of intact parasites, because antigens may still be detected. Therefore, the assay should not be used to follow patients after adequate therapy has been given. The RDT should not be viewed as a replacement for blood smears but rather as a substitute in situations in which reliable blood smears will not be readily available or when the clinical situation (eg, a “stat lab” in an emergency department) is critical and an immediate diagnosis is required. Such testing should be followed as soon as possible by microscopy with good-quality thick and thin blood films. A negative RDT result in a clinical setting suggesting malaria

must be followed up by microscopy of adequate blood smears, without delay.

LYMPHATIC FILARIASIS, LOA LOA, AND ONCHOCERCIASIS

The standard method for diagnosis of lymphatic filariasis is microscopy of thick and thin blood or buffy coat films stained with Giemsa or other appropriate stains (see “Babesiosis and Malaria” section) [1, 2]. Well-trained and experienced technologists must be available to prepare and examine these slides and to recognize these unusual organisms. The quantitative buffy coat system (which requires commercial equipment and fluorescence microscopy) may also be used to enhance sensitivity, but it is not widely available in clinical laboratories. Additional thin smears would be required to determine identification of any microfilaria present. Live motile microfilaria may also be observed in fresh wet preparations of blood or buffy coat samples. Concentration methods using centrifugation or stained polycarbonate filters can increase the sensitivity of light microscopy. *Wuchereria bancrofti* and *Brugia malayi* may have nocturnal periodicity (depending on the geographic origin of the infection), and blood may be best examined from 10 PM to 2 AM.

Serologic testing is available from referral laboratories (eg, Focus Diagnostics) and can be used to detect filarial IgG4 antibodies. Antigens of *Dirofilaria immitis* are used that can cross-react with all filarial species, so the assay is nonspecific and provides only qualitative positive or negative results. Antibodies may not be detectable in serum samples obtained from patients with chronic lymphatic filariasis (elephantiasis). Antigen-detecting immunoassays performed at the CDC are an adjunct to assays that detect microfilaremia due to *W. bancrofti*. An ICT card assay (BinaxNOW Filariasis; Binax) and an Og43C ELISA (Filariasis CELISA; Cellabs) are reported to be both sensitive and specific [12, 13], although neither of these commercial assays is approved by the FDA. Ultrasound detection of motile adult worms in major lower extremity lymphatics is another valuable diagnostic method. Living adult worms in pelvic or lower extremity lymphatics can be recognized by their size, appearance, and motility, which produces a typical “filarial dance sign” [14]. Also, adult worms of *L. loa* can sometimes be observed in the subconjunctival space of the eye, thus allowing for a presumptive diagnosis.

The standard method for laboratory diagnosis of onchocerciasis is microscopy of multiple Giemsa-stained “skin snips,” which can demonstrate microfilaria [15]. Skin snips are minute bloodless biopsies taken down to the dermal papillae using a razor blade or corneoscleral biopsy instrument. Multiple (up to 6) thin 1–2 mg snips of skin are needed. They can be taken from tissue that surrounds a nodule suspected of containing adult worms or from random body sites overlying the scapula

or iliac crest. Fresh unstained wet preparations of skin snips should also be examined after incubation in saline at 37°C for 2–24 h. Microfilaria can also be seen in histopathologic sections of skin biopsies stained with hematoxylin and eosin. An ELISA is available from referral laboratories (eg, Focus Diagnostics) that detects filarial IgG4 antibodies. Antigens of *D. immitis* are used that cross-react with all filarial species, so the assay is nonspecific and provides only qualitative positive or negative results. Other valuable diagnostic methods include ultrasonic detection, surgical recovery of adult worms from a subcutaneous nodule, and observation of microfilaria in the eye by slit-lamp exam.

VISCERAL AND CUTANEOUS LEISHMANIASIS

The standard method for laboratory diagnosis of visceral leishmaniasis is histopathologic microscopy of fixed aspirate samples of bone marrow or spleen stained with Giemsa or hematoxylin-eosin stain or biopsies of bone marrow or liver [2, 16]. Well-trained and experienced technologists must be available, because tissue amastigotes may be difficult to identify and may be hard to differentiate from other organisms (such as *Histoplasma* species). Culture of fresh specimens can be useful if media and resources are available without undue delay. Culture in Novy-MacNeal-Nicolle agar or other suitable media of aspirate samples of bone marrow or spleen or a biopsy of bone marrow or liver is preferred. Cultures are examined microscopically for motile parasites. An indirect fluorescent antibody immunoassay is available from referral laboratories (eg, Focus Diagnostics) and the CDC that detects IgG and IgM antibodies against *Leishmania donovani*, *L. tropica*, and *L. braziliensis*. An FDA-approved rapid and simple ICT “strip” assay (Kalazar Detect; InBios) is commercially available. This ICT assay detects antibodies against the rK39 antigen of *L. donovani* and is >90% sensitive and specific for visceral leishmaniasis in India but may be less accurate in other regions [16]. Sensitivity is diminished when this assay is used for immunocompromised patients (eg, patient infected with human immunodeficiency virus). These serologic assays should not be used in the diagnosis of cutaneous leishmaniasis, although cutaneous leishmaniasis has been associated with a reactive rK39 assay result in some US servicemen without clinical evidence of visceral leishmaniasis [17].

The standard method for laboratory diagnosis of cutaneous leishmaniasis and mucocutaneous leishmaniasis is microscopy of Giemsa-stained or hematoxylin-eosin-stained scrapings, aspirate samples, or biopsy samples of skin ulcers or mucosal lesions [2, 16, 18]. Subsurface needle aspirate samples or scrapings or dermal biopsies of the base or the advancing edge of a skin ulcer should be performed while avoiding the purulent center of the ulcer. Biopsy is most practical for mucosal lesions. With the addition of culture, the sensitivity of diagnosis increases to 85%. Serologic assays developed for the diagnosis of

visceral leishmaniasis are not adequately sensitive or specific for routine use in the diagnosis of cutaneous leishmaniasis. PCR methods have been described for the diagnosis of visceral leishmaniasis and cutaneous leishmaniasis in Italy, Africa, and South America but are not generally available for patient diagnostic testing [19–21].

CYSTICERCOSIS, ECHINOCOCCOSIS, AND TRICHINOSIS

The laboratory diagnosis of these 3 infections, which are caused by tissue invasion of the larval form of the parasites, is primarily serological but is supported by clinical, epidemiologic, and radiographic findings. For cysticercosis and echinococcosis, enzyme immunoassays and western blot immunoassays are available [22–24]. These assays have decreased sensitivities when lesions are calcified and presumably inactive, when only one cysticercus is present, or when echinococcal cysts are only located in organs other than the liver. There is also a high degree of cross-reactivity between assays for these 2 organisms, but this is rarely a problem because the clinical diseases and epidemiology are quite different. Microscopy of histopathologic sections of tissue biopsies or cyst aspirate samples may reveal characteristic larval cestode organisms or tissue remnants and/or hooklets.

The diagnosis of trichinosis depends heavily on clinical suspicion arising from typical clinical findings and a history of ingestion of raw or inadequately cooked pork or bear meat. The suspect meat will often have been home processed and is a good source for microscopy of muscle tissue. Eosinophilia is invariably present. A positive patient muscle biopsy will establish the diagnosis but is seldom available. A positive serologic test result is an adjunct to diagnosis for highly suspect cases, but the specificity of commercial assays is not optimal, and the clinician should be wary of false-positive results among patients with an atypical clinical picture [25]. The lack of quantitative results with the use of an ELISA can be problematic in that borderline positive results will only be recognized as positive results by clinicians. In cases in which there is a questionable ELISA result, the laboratory may be able to provide additional information indicating whether the result was borderline positive or strongly positive.

AFRICAN TRYPANOSOMIASIS AND AMERICAN TRYPANOSOMIASIS

The standard method for diagnosis of human African trypanosomiasis (or sleeping sickness, which is due to *Trypanosoma brucei* subspecies, *T. rhodesiense* and *T. gambiense*) during the acute phase of infection is microscopy of thick and thin blood or buffy coat films stained with Giemsa or other appropriate stains (see “Babesiosis and Malaria” section) [1, 2]. Well-trained and experienced technologists must be available to thoroughly

examine the entire slide, because the parasites are often few in number and may be unexpectedly detected in blood submitted for malaria diagnosis. Live motile trypanosomes may also be observed in fresh wet preparations of blood or buffy coat samples. It must be emphasized that live trypanosomes are highly infectious, and specimens must be handled with care using standard precautions, including the use of gloves and other personal protective measures. Microscopy of blood is less useful during the meningoencephalitic stages of infection because of the low level of parasitemia. Although trypanosomes are rare and difficult to detect in cerebrospinal fluid, sensitivity may be enhanced by double centrifugation of the cerebrospinal fluid before examining the sediment. Detection of Mott cells in cerebrospinal fluid is highly suggestive of human African trypanosomiasis in the appropriate clinical and epidemiologic setting [26, 27]. Serologic and/or PCR assays may be available from the CDC in the United States or from provincial laboratories in Canada. Another valuable resource for information on diagnosis of human African trypanosomiasis is the Department of Parasitology of the Prince Leopold Institute of Tropical Medicine in Antwerp, Belgium (Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium; e-mail: dir@itg.be; Web site: <http://www.itg.be>).

The standard method for diagnosis of American trypanosomiasis (or Chagas disease due to *Trypanosoma cruzi*) during the acute phase of infection is microscopy of thick and thin blood or buffy coat films stained with Giemsa or other appropriate stains (see “Babesiosis and Malaria” section) [1, 2]. Live motile trypanosomes may also be observed in fresh wet preparations of blood or buffy coat samples that must be handled with care using standard precautions, including the use of gloves and other personal protective measures, because the organisms are highly infectious. Microscopy is less useful during the latent and chronic stages of infection when levels of parasitemia are very low and the nonmotile (amastigote) intracellular form of *T. cruzi* predominates. The diagnosis during these stages may be established serologically or by microscopic examination and culture of tissue aspirate samples or biopsies [26]. Culture in easily prepared Novy-MacNeal-Nicolle agar or Evans’ modified Tobie’s medium of any appropriate blood or tissue specimen during the acute and chronic stages will add to the sensitivity of laboratory diagnosis. Serology with commercially available ELISA kits is of greatest use during the latent and chronic stages of disease [28]. Positive test results are considered evidence of active infection and would exclude blood and/or tissue donors, because the infection has been transmitted by transfusion and transplantation [29]. Rapid real-time PCR assays have recently been developed but are available only in research laboratories. Xenodiagnosis, a method whereby uninfected laboratory-reared vectors (ie, reduviid or “kissing”

bugs) are allowed to feed on patients and are then examined for trypanosomes, is not available in US clinical laboratories.

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