



Diagnostic Identification and Differentiation of Microfilariae

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ABSTRACT The morphologic similarities of the microfilariae and their infrequency in clinical specimens in settings of endemicity present challenges to clinical laboratories in maintaining competence for accurate identification and differentiation. We present here a review of the primary filarial nematodes causing human infection, including an illustrated key, which we hope will improve the diagnostic capabilities of hematologists, microbiologists, medical technologists, and similarly qualified laboratorians.

KEYWORDS *Brugia*, *Loa*, *Mansonella*, *Onchocerca*, *Wuchereria*, filariasis, microfilariae

Filariasis is an infection with nematodes in the superfamily Filarioidea. Causative agents of human filariasis include *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Loa loa*, *Mansonella perstans*, *Mansonella ozzardi*, *Mansonella streptocerca*, and *Onchocerca volvulus* (1) (Table 1). In addition, humans can become infected with several zoonotic filarial nematodes, including other *Onchocerca* and *Brugia* species, *Dirofilaria* species, and others of uncertain affiliations (2).

These nematodes share a feature of a microfilarial larval stage that is a precursor to the L1 stage (the first larval stage for most parasitic nematodes of humans). Accurate detection and identification of these microfilarial larvae are necessary for the clinical management of patients infected with filarial nematodes.

Filarial nematodes cause a variety of clinical manifestations in the human host, from asymptomatic infection to lymphedema (*W. bancrofti* and *Brugia* spp.), subcutaneous nodules or swellings (*L. loa* and *O. volvulus*), and ocular involvement (*L. loa* and *O. volvulus*). Lymphatic filariasis caused by *W. bancrofti* and *Brugia* species, as well as river blindness caused by *O. volvulus*, have a significant public health impact, with an economic burden that affects the poorest layers of society in developing countries. Advanced forms of lymphatic filariasis are also associated with mental suffering and social stigma for the affected individuals.

Clinical practices. The approach to diagnosis and treatment of filarial infections varies based on the geographic setting and access to medical resources and relies on a combination of clinical, radiological, and laboratory diagnostic methods available (https://www.cdc.gov/parasites/lymphaticfilariasis/health_professionals/index.html, https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html, https://www.cdc.gov/parasites/loiasis/health_professionals/index.html, https://www.who.int/lymphatic_filariasis/en/, and <https://www.who.int/apoc/onchocerciasis/en/>) (3). Health care professionals practicing in settings of endemicity are generally proficient in diagnosing filarial infections based on the characteristic clinical presentations of each infection. Tools such as point-of-care ultrasonography may provide additional evidence of infection when performed by an experienced practitioner (4). In settings of endemicity, the goal is to prevent the complications of long-term disease, particularly for lymphatic filariasis and onchocerciasis, and to treat the infection when possible. Significant progress has been made in recent years to control and even eliminate filarial

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TABLE 1 Causative agents of human filariasis, including their geographic distribution, preferred specimen type, periodicity, length, and diagnostic morphologic features

Species	Geographic distribution	Preferred specimen type	Periodicity	Length (μm)	Key diagnostic morphologic features
<i>Wuchereria bancrofti</i>	Tropics and subtropics of Africa, Asia, South Pacific, South America, Caribbean	Blood	Nocturnal	244–296 ^a	Usually colorless sheath (Giemsa), anucleate tail, short headspace, relatively loose nuclear column
<i>Brugia malayi</i>	Southeast Asia, including Philippines, Malaysia, Indonesia, South Korea, Vietnam, and India	Blood	Nocturnal	177–230 ^a	Usually hot-pink sheath (Giemsa), terminal and subterminal tail nuclei separated by large gaps, long headspace
<i>Brugia timori</i>	Lesser Sunda Archipelago	Blood	Nocturnal	310 (avg) ^a	Usually colorless sheath (Giemsa), terminal and subterminal tail nuclei separated by large gaps, long headspace
<i>Loa loa</i>	West Central Africa, south of the Sahara	Blood	Diurnal	231–250 ^a	Usually colorless sheath (Giemsa), tail nuclei randomly distributed to the tip of the tail, short headspace, relatively dense nuclear column
<i>Mansonella perstans</i>	Sub-Saharan tropical Africa, Central and South America, Caribbean	Blood	None	190–200 ^a	Sheath never present, compact nuclear column, tail nuclei densely packed to tip of blunt tail
<i>Mansonella ozzardi</i>	Central and South America, Caribbean	Blood	None	163–203 ^a	Sheath never present, compact nuclear column, anucleate tail, tail tapered and pointed
<i>Mansonella streptocerca</i>	Tropical sub-Saharan Africa	Skin snips	None	180–240 ^b	Sheath never present, hooked tail with nuclei arranged to the tip of the tail
<i>Onchocerca volvulus</i>	Western and central Africa south of the Sahara, Yemen, Central and South America	Skin snips	None	304–315 ^b	Sheath never present, tail tapered and often flexed, anucleate

^aIn stained blood films.

^bIn skin snips.

infections in settings of endemicity through the use of annual or biannual mass drug administration (MDA) using the donated anthelmintics albendazole, diethylcarbamazine (DEC), and ivermectin (https://www.who.int/lymphatic_filariasis/en/ and <https://www.who.int/apoc/onchocerciasis/en/>). The principle of MDA is to deliver appropriate chemotherapy to all individuals in a susceptible population without upfront testing to identify infected individuals. When available, laboratory diagnosis and specific treatment are also utilized. In settings nonendemic for the infections, such as the United States, the diagnosis of a filarial infection may be missed if a travel history is not obtained and the clinical team is not familiar with the signs and symptoms of infection. The aforementioned challenges of laboratory diagnosis in settings of nonendemicity may also contribute to delays in diagnosis. State and other local public health laboratories may offer diagnostic assistance which might provide improved turnaround time. The U.S. Centers for Disease Control and Prevention (CDC) DPDx service (<https://www.cdc.gov/dpdx/index.html>) provides free, rapid telediagnostic consultative services to assist local diagnostic and public health laboratories in diagnosing challenging parasitic cases, and CDC subject matter experts are available for consultation with laboratorians and clinicians on diagnostic and treatment options. Their website also offers a comprehensive image library and downloadable bench aids for the diagnosis of filarial infections and other parasitic diseases.

Biology. All human filarial nematodes have a similar pattern to their life cycles, and it is helpful to have an understanding of the life cycle to appreciate several key diagnostic aspects of infection. Adults reside in various tissues of the definitive host, including the lymphatics (*W. bancrofti* and *Brugia* spp.), subcutaneous tissue (*L. loa*, *Onchocerca* spp., *M. ozzardi*, and *M. streptocerca*), and peritoneal and pleural cavities (*M. perstans*). Adults of some species may migrate to the eyes (*L. loa*). Mated females release microfilariae into the blood or surrounding skin, where they are picked up by an appropriate insect vector while taking a blood meal. A variety of blood-feeding flies serve as vectors of filarial nematodes, including mosquitoes (*W. bancrofti* and *Brugia* spp.), biting midges (*M. ozzardi*, *M. perstans*, and *M. streptocerca*), black flies (*M. ozzardi* and *O. volvulus*), and deer flies (*L. loa*). Microfilariae penetrate the midgut of the vector and migrate to the musculature where they develop to infectious L3 (third-stage, or filariform) larvae. L3 larvae migrate to the fly's mouthparts and infect a new host when the vector takes a blood meal (<https://www.cdc.gov/dpdx/index.html>).

SPECIMEN COLLECTION, PREPARATION, AND EXAMINATION

Blood. The traditional method of diagnosis of most filarial nematode infections continues to be detection of microfilariae on stained thick and thin blood films. Proper collection and processing of blood specimens are essential for a reliable diagnosis. The notable exception to the use of blood specimens is for the diagnosis of onchocerciasis and *M. streptocerca* infection, which involves examination of skin snips (see below).

(i) Periodicity. The microfilariae of some species of filarial nematodes exhibit periodicity in which they circulate in the blood predominately at certain times of the day. The time of circulation corresponds with the primary times that the insect vector takes its blood meal; thus, this periodicity aids in the continuation of the parasite's life cycle (5). For more sensitive detection of *Wuchereria bancrofti*, *Brugia* spp., and *L. loa*, it is important to understand the optimal times their microfilariae will be circulating in peripheral blood and obtain blood specimens during these time periods.

Wuchereria bancrofti and the *Brugia* species exhibit nocturnal periodicity, and the optimum time for blood collection is between 10:00 p.m. and 2:00 a.m. (1), although there is a subperiodic strain of *W. bancrofti* in the South Pacific whose peak times are between 12:00 p.m. and 6:00 p.m. (6).

Loa loa exhibits diurnal periodicity, and peak times for microfilarial detection are between 10:00 a.m. and 2:00 p.m. (1, 5).

Mansonella perstans and *M. ozzardi* do not exhibit specific periodicity, and thus, blood can be drawn at any time for the detection of peripherally circulating microfilariae.

(ii) Smear preparation. Thick and thin blood films used for the detection of other blood parasites are generally adequate for the detection of microfilariae, although concentration methods provide increased sensitivity (<https://www.cdc.gov/dpdx/index.html>) (also, see below). Blood may be collected by the fingerstick method or in EDTA but should be processed as soon as possible to ensure an accurate diagnosis. Delay in processing might alter the morphology of some species, most notably, the loss of the sheath, which could result in the misidentification of a normally sheathed species, such as those of *Mansonella*. Thick blood films allow for greater sensitivity due to the higher concentration of blood elements, and unlike with *Plasmodium* species, identification of microfilariae to the species level can be performed as easily with the thick film as with the thin. Thick films can be made with a volume of blood from as little as 20 μ l to 60 μ l (preferred) (7). It is recommended that at least two thin and two thick films should be examined (<https://www.cdc.gov/dpdx/index.html>); however, it is our experience that more than two of each type should be examined when microfilariae are suspected or that a concentration method (e.g., Knott's) be used.

(iii) Staining. The recommended staining methods for the detection of microfilariae in blood are Giemsa, Wright-Giemsa, and Delafield's hematoxylin (7, 8). Ahad (9) proposed a rapid method using Ziehl-Neelsen carbol fuchsin followed by Leishman's stain.

(iv) Concentration procedures. Concentration procedures, such as the Knott's technique and membrane filtration technique, can be used for the enhanced detection of microfilariae in blood or other body fluids (7, 8). The Knott's technique is preferred when the microfilarial load is low. The use of quantitative buffy coat has been reported to be acceptable for the diagnosis of microfilariae, with a sensitivity similar to that of a thick film (10).

Knott's procedure (adapted from reference 7):

1. Collect 1 ml of whole blood or blood in citrate or EDTA by venipuncture and place in a centrifuge tube with 10 ml of 10% formalin. Shake vigorously to aid in lysing of the erythrocytes.
2. Centrifuge at $300 \times g$ for 2 minutes. If a centrifuge is not available, place the tube in an upright position for 12 hours for gravitational sedimentation.
3. Pour off the supernatant.
4. Examine a drop of sediment on a slide with a coverslip with the 10 \times objective.
5. A drop of sediment may be spread onto a slide, allowed to dry, and stained with Giemsa or hematoxylin per normal procedures.

Membrane filter procedure (adapted from references 1 and 7):

1. Collect fresh blood in sodium citrate or EDTA.
2. Add 1 ml of the blood to 10 ml of 10% Teepol-saline solution (50 g Teepol concentrate to 450 ml saline).
3. Place a 25-mm Nuclepore filter of 5- μ m porosity over a 25-mm supporting filter paper moistened with water securely into a filter holder.
4. Remove plunger from the barrel of a 20-ml syringe and connect the barrel of the syringe to the filter holder.
5. Pour the blood-Teepol mixture (see step 2) into the barrel of syringe, replace the plunger in the syringe, and gently force the solution through the filter.
6. Remove the syringe from the filter holder, draw up 10 ml of water into syringe, reattach the filter holder, and gently wash the filter by flushing the water through it. Do this three times total.
7. Pass 3 ml of methanol through the filter to fix the microfilariae.
8. Remove the filter from the holder and place on a glass slide; allow to dry thoroughly.
9. Stain with Giemsa or preferred stain per the normal procedure.
10. Dip the slide in toluene to avoid air bubble formation.
11. Add a drop of mounting medium and a coverslip.
12. Examine the slide when dry.

(v) Microscopy. Because microfilariae are large, they can be detected by screening at a lower magnification (e.g., $\times 10$) and then examined at a higher magnification ($\times 40$, $\times 50$ with oil, or $\times 100$ with oil) for species-level identification. Because of the large size of microfilariae and rarity in clinical specimens, it is imperative that the entire blood film should be scanned with a $10\times$ objective before reporting as negative; higher power, including oil immersion, should then be used to screen for malaria and other blood pathogens.

Skin snips. Skin snips are used for the detection of *Mansonella streptocerca* and *Onchocerca* species. Snips should be thin, including just the epidermis and superficial dermis, and collected with minimal bleeding to avoid contamination of the specimen with peripherally circulating microfilariae (particularly in areas of endemicity where coinfections are common) (<https://www.cdc.gov/dpdx/index.html>). The skin snips are generally collected from several sites on the body to maximize detection sensitivity; common locations include the lower extremities and the skin over bony prominences of the iliac crest and scapula (https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html). Surveys have demonstrated that when the microfilarial density is high, two snips can be adequate, but if the density is low, using six snips at multiple sites improves the sensitivity. Microfilariae in skin snips do not exhibit periodicity and thus can be obtained at any time (https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html). The following is a procedure for obtaining skin snips. A sclerocorneal biopsy may also be used to obtain diagnostic skin specimens (https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html).

Biopsy procedure (adapted from reference 7):

- Using a sterile needle, raise a small region of skin (e.g., 3.0 mm in diameter) slightly and then use a sterile razor blade to shave off the raised area.
- Transfer the skin biopsy specimen to a small tube or well of a 96-well or similarly sized plate containing normal saline, distilled water, or tissue culture medium.
- Examine the skin and saline after 30 minutes to 3 hours, looking for motile microfilariae. If negative, continue incubating overnight at 37°C .
- Place aliquots of liquid from positive specimens on a microscopy slide, allow to dry, and then stain with Giemsa, Wright-Giemsa, or hematoxylin for species-level identification.

Other specimen types. Rarely, microfilariae may be identified in other body fluids. Microfilariae of *O. volvulus* have been found in urine and sputum samples after the administration of DEC (11), and microfilariae of *W. bancrofti* have been identified in the urine of patients infected with chyluria, as well as in the hydrocele fluid (11, 12).

Urine and hydrocele fluid specimens should be centrifuged for 5 min at $350\times g$ or more; if microfilariae are present, they may be detected in the sediment (7). Sputum specimens are processed per normal cytological specimens (8).

Urine, hydrocele fluid, and sputum may be fixed and stained as with blood specimens (7).

MORPHOLOGIC IDENTIFICATION

General considerations. Morphologic detection of microfilariae in blood or skin snips continues to be the gold standard for the routine clinical diagnosis of most human filariases. There are several important factors to consider when attempting an identification of microfilariae to the genus or species level. Obtaining a detailed patient travel history is very important, as it can help rule out certain filariases depending on where the patient has traveled to or is native from. Important morphologic criteria include the length and width of the microfilarial body, presence or absence of a sheath, and arrangement of nuclei in the tail (1, 8, 13). There are several published atlases and bench guides to aid in the identification of microfilariae (1, 7, 13) (Fig. 1).

(i) Size. Determining the approximate body length and width is an important step in the identification of microfilariae in blood. Determining the specific length can be difficult if the worm is bent or coiled, but getting a general idea of the size can be

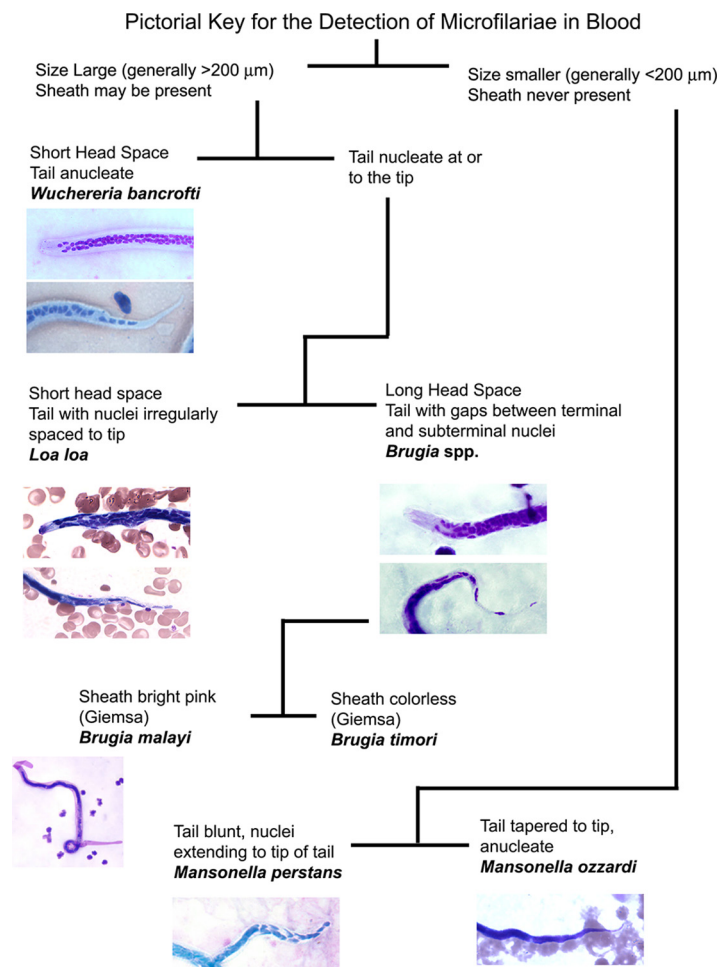


FIG 1 Illustrated key to the microfilariae observed in blood specimens.

helpful for eliminating some species. Digital image analysis with appropriately calibrated measurement software can overcome this challenge if available to the laboratory. Microfilariae in blood can be placed into one of two groups based on general size, the large species (*W. bancrofti*, *L. loa*, and *Brugia* spp.) and the small species (*Mansonella* spp.). The large species are as wide as or wider than a normal red blood cell (RBC; 6 to 8 μm in diameter), while the width of the small species is on average half the diameter of a normal RBC. On a thick film, the large species may appear as wide as the nuclear structure of leukocytes, whereas the *Mansonella* species are noticeably thinner than any of the host blood cells.

Length and width can also be very helpful features for separating *M. ozzardi* and *Onchocerca* species in skin snips.

(ii) Sheath. The presence or absence of a sheath, and the color which it stains with Giemsa, can be a helpful, but often misleading, feature. On blood films processed in a proper and timely manner, *W. bancrofti*, *Brugia* spp., and *L. loa* may possess a sheath, while *Mansonella* spp. are intrinsically unsheathed. If there is a substantial delay in processing, the sheathed species may shed their sheaths, and the sheaths may not be visible via microscopy; therefore, the absence of a sheath in itself is not indicative of the presence of *Mansonella* spp. Further, retraction or shrinkage of microfilariae in a blood smear may create the impression of a sheath when one is not there, which can cause diagnostic uncertainty. Thus, the sheath is only one feature that should be considered when undertaking morphologic assessment of microfilariae on Giemsa-stained blood films and should always be viewed in the context of the size of the organisms.

On a Giemsa-stained blood slide, the sheath of *W. bancrofti*, *B. timori*, and *L. loa* will appear colorless, but the sheath of *B. malayi* will usually stain bright pink (1). This can vary based on stain and pH, and in rare cases, *W. bancrofti* and *L. loa* have presented with a pink sheath (B. A. Mathison, unpublished observations).

Filarial nematodes in skin snips do not possess a sheath.

(iii) Nuclear column. The arrangement of nuclei of the microfilariae, specifically, the relative length of the headspace and the arrangement of nuclei at the tip of the tail, are the most important diagnostic features for a genus- or species-level identification.

Pitfalls and mimics. Few artifacts may be confused with microfilariae. Airborne contamination with the conidia of *Helicospirium* spp. and similar fungi may be mistaken for microfilariae, but they are significantly smaller than true microfilariae and lack associated internal nuclei (14). Synthetic fibers may also be mistaken for microfilariae; however, the absence of internal features should be an immediate indication of such artifacts. Finally, free-living nematodes may contaminate buffers or laboratory supplies and may be mistaken for parasitic species (15). These nematodes may be more difficult to differentiate from human-infecting filariae but may be noted on all slides prepared with the contaminated lot reagents, which should prompt a more thorough investigation.

OTHER DIAGNOSTIC TESTS

Molecular methods. Molecular assays are not routinely available for the diagnosis of filariasis in most clinical or reference labs but may be available at specialized research centers and public health labs. Several assays have been developed but are used primarily for research or epidemiologic investigations (see also individual species treatments below). Such assays have been described using real-time PCR in the laboratory, as well as using loop-mediated isothermal amplification (LAMP). Real-time PCR is primarily of benefit to laboratories in developed countries that have adequate laboratory infrastructure and for which cost is less of a prohibitive metric, while LAMP assays are an attractive option for developing countries due to the lower cost of reagents (compared to real-time PCR) and potential for near point-of-care application. To date, LAMP assays targeting various filarial nematodes (e.g., *L. loa*, *Onchocerca* spp., *Brugia* spp., and *Wuchereria* spp.) (16, 17) have been described but are not commercially available in the United States. Recently, deep-sequencing techniques have been evaluated for use in detecting filarial nematodes in the blood, along with other blood-borne parasites (18). DNA sequencing analysis approaches have previously been used to target a small subset of conserved housekeeping genes (e.g., 5S rRNA, spiced leader [SL], and 18S rRNA), which generally allows identification to the species level, thus making whole-genome analysis not essential for the identification of filarial nematodes, although it may be useful for certain species.

Antibody detection. Antibody detection assays have selected applications in the diagnosis of filariasis (see individual sections below) but are available primarily for lymphatic filariasis caused by *W. bancrofti* or *B. malayi*. Serologic assays are not available for routine diagnosis or confirmation of *L. loa*, *Mansonella* spp., and *O. volvulus* in the United States.

Rapid diagnostic tests. Immunochromatographic tests (ICTs) have been developed for lymphatic filariasis caused by *W. bancrofti* and *B. malayi* and onchocerciasis caused by *O. volvulus*. These tests are used primarily for epidemiologic investigations and for monitoring progress in elimination studies, and they are not used for routine clinical diagnosis. None of these tests are cleared by the U.S. Food and Drug Administration (FDA) for clinical use (see also under species-specific information below).

SPECIES-SPECIFIC INFORMATION

***Wuchereria bancrofti*.** (i) **Geographic distribution.** Of all of the human filarial nematodes, *W. bancrofti* has the widest geographic distribution, causing lymphatic

filariasis in the tropics and subtropics of South America, the Caribbean, Africa, Asia, and the South Pacific (https://www.who.int/lymphatic_filariasis/en/).

(ii) Morphologic identification. Microfilariae of *W. bancrofti* are 244 to 296 μm long by 7.5 to 10.0 μm wide in stained blood films. They possess a relatively short headspace, and the tail is anucleate and tapers to a point. In general, the nuclear column is relatively loose, and individual nuclei can be visualized throughout the column. In properly preserved specimens, a sheath is usually present and does not stain pink with Giemsa stain (1).

(iii) Differential diagnoses. Geographically, *W. bancrofti* overlaps several other medically important species of filarial nematodes, and as such, a critical evaluation of all morphologic criteria (especially size and nuclear arrangement) should be done to ensure a correct diagnosis.

(iv) Other diagnostic methodologies. *Wuchereria bancrofti* can also be diagnosed by antibody detection, including an enzyme-linked immunosorbent assay (ELISA) using the Bm14 and BmR1 antigens. These assays have reported 91% and 45% sensitivities, respectively, for *W. bancrofti*, but have higher sensitivities (96% and 100%, respectively) for *B. malayi* (19). These assays also exhibit some cross-reactivity with antigens of *O. volvulus* and *L. loa*. Another target used in research use only (RUO) ELISAs is Wb123, which is an antigen from L3 larvae of *W. bancrofti* (20). When used for IgG or IgG4 detection, sensitivity was 98 to 100% for *W. bancrofti*; however, specificity was higher with IgG4 (94 to 100%) than with IgG (84 to 100%) (20). This specificity improvement was primarily attributed to IgG4 not cross-reacting with *L. loa* and *O. volvulus*, which was seen when detecting IgG. Wb123 is the target antigen used in the commercially available (RUO) ELISA from InBios International, Inc. (Seattle, WA). These reagents are currently used as a laboratory-developed test with unpublished analytical and clinical performance.

The BinaxNow filariasis ICT (Abbott, Chicago, IL) has been shown to be effective for diagnosis and evaluation for elimination programs in areas of endemicity (21); however, this test is not currently approved for diagnostic testing in the United States. PCR assays targeting the pWb12 repetitive regions are available for *W. bancrofti* (22–25), but they are primarily used in epidemiological surveys and elimination programs and not for routine clinical diagnosis. None of the aforementioned tests are cleared by the FDA for clinical diagnosis.

***Brugia malayi.* (i) Geographic distribution.** *Brugia malayi* causes lymphatic filariasis in Southeast Asia, including the Philippines, Malaysia, Indonesia, South Korea, Vietnam, and India (https://www.cdc.gov/parasites/lymphaticfilariasis/health_professionals/index.html and https://www.who.int/lymphatic_filariasis/en/).

(ii) Morphologic identification. Microfilariae of *B. malayi* are 177 to 230 μm long by 5 to 6 μm wide in stained blood films. They possess a longer headspace and a more compact nuclear column than *W. bancrofti*. The tail tapers to a point and possesses both a terminal nucleus and a subterminal nucleus, with significant gaps between these nuclei and the nuclear column. In properly preserved specimens, a sheath is usually present that typically stains bright pink with Giemsa stain (1).

(iii) Differential diagnoses. In Southeast Asia, *B. malayi* is most morphologically similar to *W. bancrofti* and *B. timori*. The three species clinically manifest similarly and are treated similarly. *Brugia malayi* can be separated from *W. bancrofti* by the length of the headspace and characteristics of the tail nuclei. If a sheath is present and stained with Giemsa stain, the pink sheath of *B. malayi* can often be distinguished from the colorless sheath of *W. bancrofti*. The morphologic features of *B. malayi* and *B. timori* are nearly identical, and in the absence of precise travel history, the best features to separate the two are mean length and the color of the sheath.

(iv) Other diagnostic methodologies. *Brugia malayi* can also be diagnosed serologically by ELISAs targeting the Bm14 and BmR1 antigens. These assays have 96% and 100% sensitivity, respectively, for *B. malayi*, as well as 94% and 46% sensitivity, respectively, for *W. bancrofti*. While they can also cross-react with *O. volvulus* and *L. loa*,

these two species do not occur in the same geographic areas as *B. malayi*. PCR assays are available for *B. malayi*, but they are not commonly used for routine clinical diagnosis (23, 26). Recently, an unbiased metagenomic approach was used to identify *B. malayi* to the species level, which may provide future utility for whole-genome sequencing when targeted gene sequencing fails to differentiate between species (27). Clinically, this level of differentiation is not required, or is not financially justified, and is more academically/epidemiologically applicable.

***Brugia timori*. (i) Geographic distribution.** *Brugia timori* is restricted to several islands in the Lesser Sunda Archipelago of eastern Indonesia, including Timor, Sumba, Lembata, Pantar, and Alor (28).

(ii) Morphologic identification. Microfilariae of *B. timori* are similar to those of *B. malayi* but are larger, having a mean length of 310 μm and a width of 6 to 7 μm . The long headspace and arrangement of tail nuclei are similar between the two species. Unlike with *B. malayi*, the sheath of *B. timori* does not stain bright pink with Giemsa stain (1).

(iii) Differential diagnoses. *Brugia timori* and *B. malayi* are allopatric in distribution, and they do not occur together (28); however, morphologically, they are very similar. The best way to separate them is mean length and color of the sheath when stained with Giemsa stain at a pH of ± 7.0 . On some islands, *B. timori* and *W. bancrofti* occur together, whereby they can be separated by characteristics of the headspace and tail nuclei.

(iv) Other diagnostic methodologies. Serologic assays utilizing the Bm14 and BmR1 antigens for lymphatic filariasis have not been sufficiently evaluated for *B. timori* to determine the degree of cross-reactivity. PCR assays for *B. malayi* often cross-react with *B. timori*, and the best molecular method for separating them is random fragment length polymorphism (RFLP) analysis of the mitochondrial cytochrome *c* oxidase subunit 2 (28).

***Loa loa*. (i) Geographic distribution.** *Loa loa* has a limited geographic distribution, currently found only in tropical regions of west central Africa, south of the Sahara (https://www.cdc.gov/parasites/loiasis/health_professionals/index.html). Reports of *L. loa* from India should be viewed with caution, as this species does not naturally occur outside Africa; such cases probably represent misidentification of *Dirofilaria repens*, which causes clinically similar ocular infections in Europe and Asia.

(ii) Morphologic identification. Microfilariae of *L. loa* are 231 to 250 μm long in stained blood films. The sheath, when present, does not stain with Giemsa stain. The microfilariae have a relatively short headspace, and the tail is nucleate, with the nuclei irregularly arranged to the tapered tip (1).

Quantification of the *L. loa* microfilariae in peripheral blood may be important for treatment purposes, as the drug of choice (DEC) has been associated with severe neurologic manifestations, including fatal encephalopathy, in cases of high microfilariaemia ($\geq 8,000$ microfilariae per ml of blood). Therapeutic apheresis may be used in these cases to reduce the microfilarial load prior to initiating DEC treatment (https://www.cdc.gov/parasites/loiasis/health_professionals/index.html).

(iii) Differential diagnoses. Where it is endemic, microfilariae of *L. loa* should be differentiated from *W. bancrofti* and *M. perstans*. Compared to *W. bancrofti*, *L. loa* microfilariae can be recognized by having nuclei extending to the tip of the tail, and compared to *M. perstans*, they may be recognized by being much larger, usually possessing a sheath and having a tapered tail. Adults of *L. loa* removed from the eye or skin need to be differentiated from those of *Dirofilaria* species [see "*Loa loa*. (iv) Other diagnostic methodologies," below].

(iv) Other diagnostic methodologies. *Loa loa* may also be diagnosed by the finding of adult worms in the eye or extracted from migratory subcutaneous swellings (Calabar swellings). Adults from these sites need to be distinguished from adults of *Dirofilaria*. Adult female *L. loa* worms are 50 to 70 mm long; the males are smaller, at 30 to 35 mm long. Adults of both sexes have randomly arranged cuticular nodules called

bosses, which distinguish them from *Dirofilaria* species that normally possess longitudinal cuticular ridges (29, 30). There are no routine serologic nor molecular tests available for diagnosing loiasis in the United States. One aim of molecular testing has been to develop a rapid quantitative or semiquantitative assay capable of determining *L. loa* microfilaremia in advance of drug therapy to avoid the complications described above. Though proof-of-concept assays using loop-mediated isothermal amplification (LAMP) have shown *in vitro* success, implementation in clinical use might be challenged by cost, since this technology would be desired in countries of endemicity, rather than in countries where travelers may be returning with *L. loa* and for which standard diagnostic procedures are readily available (17).

***Mansonella perstans*. (i) Geographic distribution.** *Mansonella perstans* is native to much of Africa south of the Sahara, except for extreme southern Africa and several surrounding islands (31). The species has been introduced to the New World, where it occurs in Central and South America and on several Caribbean islands (1, 29).

(ii) Morphologic identification. Microfilariae of *M. perstans* measure 190 to 200 μm long in stained blood films. Microfilariae lack a sheath and are aperiodic. They possess a compact nuclear column that contains nuclei to the tip of the bluntly rounded tail (1).

(iii) Differential diagnoses. In parts of Africa, *M. perstans* needs to be differentiated from the much larger *W. bancrofti* and *L. loa*; however, coinfections may occur with either of these species. In the Democratic Republic of the Congo, *M. perstans* is morphologically similar to *Microfilaria semiclarum*, a species of presumed zoonotic origin, the adult of which has yet to be described (2). Microfilariae of *M. semiclarum* are about 220 μm long by 5.0 μm wide and have approximately the middle third of the body devoid of nuclei.

In the Americas, *M. perstans* is most similar to *M. ozzardi*, which has a tapered, anucleate tail. It is important to confirm the size and nuclear structure of microfilariae before reporting them as a *Mansonella* sp.; the lack of a sheath alone is not confirmatory for this genus, as the sheath may be lacking in clinical specimens for those species that normally possess them.

(iv) Other diagnostic methodologies. There are no routine serologic or molecular tests available for *M. perstans* in the United States.

***Mansonella ozzardi*. (i) Geographic distribution.** *Mansonella ozzardi* is native to the New World and ranges from Central to South America and in the Caribbean (32).

(ii) Morphologic identification. Microfilariae on stained blood films are 163 to 203 μm long and always lack a sheath. They have a dense nuclear column which falls short of the slender, pointed tail (1).

(iii) Differential diagnoses. The only other human filarial nematode in blood that comes close to *M. ozzardi* in size is *M. perstans*, which had a bluntly rounded tail that contains a dense column of nuclei to the tip.

(iv) Other diagnostic methodologies. Primary detection by use of real-time PCR has been described in Brazil and showed significantly higher sensitivity than examination of thick blood films, suggesting that this could be a valid modality for *M. ozzardi* detection (33, 34). These assays have not been validated for clinical diagnosis in an area of nonendemicity, such as the United States.

***Mansonella streptocerca*. (i) Geographic distribution.** *Mansonella streptocerca* is endemic to tropical areas of West Central Africa (1, 32).

(ii) Morphologic identification. Microfilariae are detected in skin skips and are unsheathed, measuring 180 to 240 μm in length. Nuclei extend to the tip of the tail, which is usually bent into a hook-like shape (1) (Fig. 2).

(iii) Differential diagnoses. In areas where the geographic distribution overlaps that of *O. volvulus*, microfilariae of *M. streptocerca* need to be differentiated therefrom. Microfilariae of *O. volvulus* are larger, measuring 304 to 315 μm in length, and have a pointed, anucleate tail that is not bent into a hook-like shape.

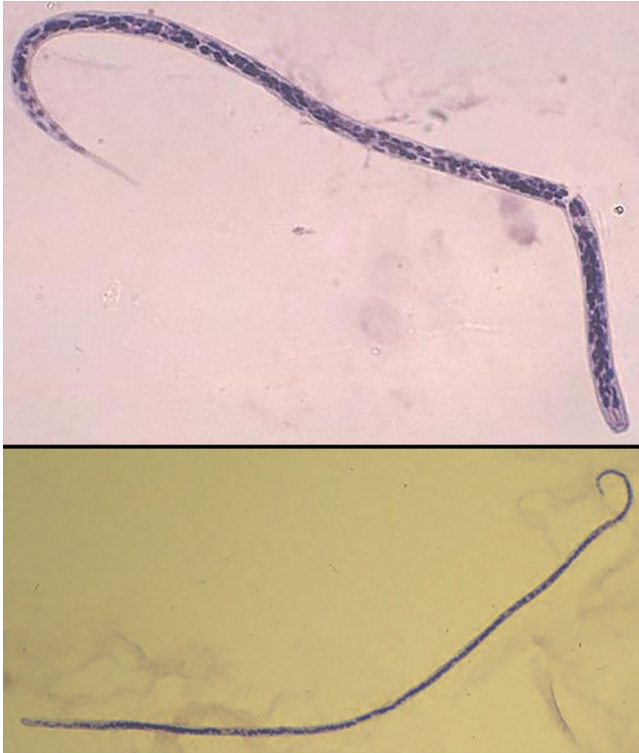


FIG 2 Morphologic comparison of *Onchocerca volvulus* (top) and *Mansonella streptocerca* (bottom) in skin specimens. Images courtesy of the CDC-DPDx.

(iv) Other diagnostic methodologies. PCR-based tests are available for *M. streptocerca*, but they are used largely for epidemiologic investigations and are not used for routine clinical diagnosis (35).

***Onchocerca volvulus*. (i) Geographic distribution.** *Onchocerca volvulus* is endemic to West and Central Africa south of the Sahara, as well as parts of eastern Africa and Yemen. In the Americas, it historically has occurred in Mexico, Guatemala, Venezuela, Colombia, Ecuador, and Brazil (1). However, elimination programs appear to have successfully ended transmission in Mexico, Colombia, and Ecuador.

(ii) Morphologic identification. Microfilariae are detected in skin snips and measure 304 to 315 μm in length. The tail tapers to an anucleate tip, which is often sharply flexed (1) (Fig. 2).

(iii) Differential diagnoses. Microfilariae of *O. volvulus* in skin snips need to be differentiated from those of *M. streptocerca*, which are smaller and have the nuclear column extending all the way to the tip of the tail. In North America, Europe, and Asia, there are cases of zoonotic onchocerciasis caused by species other than *O. volvulus*, most notably, *Onchocerca lupi* in North America and Europe (36). Separation of *O. volvulus* from zoonotic *Onchocerca* species is usually achieved by morphological identification of adult worms in biopsy specimens in conjunction with a thorough epidemiological investigation.

(iv) Other diagnostic methodologies. Onchocerciasis can also be diagnosed by identifying adults in skin biopsy specimens. There is marked sexual dimorphism, with adult females being 270 to 400 μm in diameter, while males are 130 to 210 μm in diameter. Both sexes can be identified by having a thick cuticle, coelomyarian musculature with few cells per quadrant, and a simple intestine. Females will have paired uterine tubes that will often contain microfilariae in mated, gravid females. Males have a single, coiled testis that usually contains spermatozoa (30, 37). Adults in skin biopsy specimens need to be differentiated from other nematodes that may present in subcutaneous nodules, most notably *Dirofilaria* spp. Adults of *Dirofilaria* in tissue

have taller and more numerous muscle cells and often have external longitudinal cuticular ridges and an internal cuticular ridge running along the base of the lateral chords (29, 30, 37).

Antibody detection for onchocerciasis is available but is used primarily for research purposes and elimination programs outside the United States; the test is not approved for routine clinical diagnosis in the United States. Two of the more common tests are the OV-16 antibody test (38) and the OV luciferase immunoprecipitation system (39). There are no specific molecular assays that target *O. volvulus* for routine clinical diagnosis; however, PCR amplification and sequencing of the *cox-1* gene has been used in clinical care in Australia to diagnosis onchocerciasis in specimens containing a worm visualized by microscopy (40).

Mixed infections. Mixed infections with more than one filarial nematode can be common in areas of endemicity. In Africa, for example, mixed infections usually involve *M. perstans* and another species (32). It is important to assess each patient for potential coinfections based on their geographic exposures, as the presence of more than one filarial species may present a diagnostic challenge, and when *L. loa* is present, it may change the therapeutic options. Patients with high levels of *L. loa* microfilaremia are at risk of severe neurologic manifestations following ivermectin or DEC administration; thus, these drugs should not be administered therapy for other filarial infections in the presence of *L. loa* coinfection without first consulting an expert in infectious diseases or tropical medicine (https://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html). Similarly, ivermectin cannot be safely provided as a component of mass drug administration for onchocerciasis elimination without first assessing for *L. loa* infection and associated levels of microfilaremia. Therefore, the risk of coinfection with *L. loa* may limit both therapeutic and elimination efforts. It should be noted that patients can have mixed infections with a filarial nematode and *Plasmodium* sp. (malaria) as well.

CONCLUSIONS

Filarial nematodes are found in many regions of the tropics and subtropics worldwide and are responsible for significant human morbidity. As such, it is important for diagnostic laboratorians in both settings of endemicity and nonendemicity to be familiar with the different filarial nematodes and their diagnostic features, as well as appropriate specimen collection, preparation, and examination techniques. A careful algorithmic approach (as shown in Fig. 1) may be used for simple and reliable identification. Resources such as the CDC-DPDx and experienced reference laboratories can provide assistance when needed.

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