Review

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Expanding the MDx toolbox for filarial diagnosis and surveillance

Andy Alhassan, Zhiru Li*, Catherine B. Poole*, and Clotilde K.S. Carlow

Division of Genome Biology, New England Biolabs, Ipswich, MA 01938, USA

Filarial parasites are tissue-dwelling nematodes responsible for some of the most important neglected tropical diseases. All are transmitted by blood-sucking arthropod. Onchocerciasis and lymphatic filariasis in particular are the cause of much disfigurement and morbidity. Accurate parasite detection is essential for the success of filariasis control programs. The current toolbox for diagnosis and surveillance is limited because many of the available tools suffer from lack of sensitivity and specificity, and/or are cost-prohibitive. We review the methods currently in use and discuss the prospects for developing new molecular diagnostic (MDx) tools based on nucleic acid detection. We briefly describe recent developments in isothermal nucleic acid amplification and detection, and focus on emerging technologies that are field-deployable or suitable for low-resource settings.

The need for new tools to detect filarial parasites

Filariasis is a parasitic infection caused by any one of several tissue-dwelling, filarial nematodes. The parasites are highly prevalent in regions of Africa, Asia, South and Central America, and the Yemen peninsula and are responsible for some of the most important neglected tropical diseases. Despite this, filariasis has attracted relatively little research funding compared to HIV/AIDS, tuberculosis, and malaria. Several species of filariae exist in humans and elicit disease based on their tissue localization: subcutaneous (*Onchocerca volvulus*, *Loa loa*, *Mansonella streptocerca*, and *Mansonella ozzardi*), lymphatics (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*), and body cavity (*Mansonella perstans*) (Table 1). Onchocerciasis (caused by *Onchocerca volvulus*) and lymphatic filariasis (due to *Wuchereria bancrofti* or *Brugia spp.*) in particular are the cause of much disfigurement and morbidity, resulting in social stigma and severe economic consequences. In Africa alone, more than 25 million people have onchocerciasis, or 'river blindness', and worldwide 40 million suffer from lymphatic filariasis (LF), also known as elephantiasis (www.who.int/mediacentre/factsheets/fs102/en) [1]. Most of the clinical manifestations are due to adult worms (*W. bancrofti*, *B. malayi*, and *L. loa*). Long-lived (up to 17 years), adult females are viviparous, shedding thousands of first-stage larvae, known as microfilariae (mf), into the blood. *M. streptocerca* and *O. volvulus* are exceptions because mf are present in the skin. In the latter case, mf migrate through the skin and eyes causing severe dermatitis and blindness. All filarial parasites are transmitted by the bite of a blood-sucking arthropod or vector (Table 1). mf are ingested by insects during feeding and undergo two molts to become infective third-stage larvae (L3) that are transmitted to the human host during subsequent bloodmeals. In the human host, larvae molt twice to reach sexual maturity (Figure 1) [2–5].

Accurate parasite detection is essential for the success of any filariasis control program. Current control is largely based on annual or semi-annual distribution of the larvicidal (see Glossary) compound ivermectin (Mectizan, Merck) together with albendazole (where onchocerciasis is endemic) or diethylcarbamazine citrate (where onchocerciasis is not present) to the population irrespective of infection status

Glossary

Adulticide: a drug which targets and kills the adult worm rather than an immature stage (larva).
Amicrofilaremia: absence of microfilariae (first larval stage) in the blood of filarial infected individuals.
Enzyme-linked immunosorbent assay (ELISA): a common laboratory technique used to measure the presence and abundance of antibodies or antigens in a specimen.
Immunochromatography card test: an immunoassay that qualitatively tests fingerprick blood for *Wuchereria bancrofti* circulating antigen with a visual readout.
Lateral-flow strip immunoassay: an immunoassay used to measure the presence and abundance of antibodies or antigen. The test sample migrates through a series of capillary beds, such as porous paper or polymer containing fixed reagents, resulting in a visual readout.
Larvicidal: a drug which targets and kills the immature stage (larva) of a parasite.
Mass drug administration (MDA): drug administered at regular intervals to a population at risk of infection irrespective of individual infection status.
Mazzotti test: a diagnostic test for onchocerciasis that involves administering a dose of diethylcarbamazine (50 or 100 mg). An infected individual will develop an acute rash in 2–24 h as a result of the death of microfilariae in the skin.
Microfilaria: first larval stage of filarial parasites released by adult females into blood or skin.
O-150 sequence: an *Onchocerca*-specific, highly-repetitive DNA sequence used for molecular diagnosis.
Sheath: a modified eggshell surrounding the microfilaria of particular filarial species.
Self-digitization chip: an inexpensive and simple stationary water-in-oil nanoliter reactor to quantify the amount of DNA in a sample.
Skin snip: a small skin sample (3–5 mg) collected from multiple sites (iliac crests, shoulder blades) using a razor blade or skin punch. The emergence of microfilariae from skin snips is diagnostic for infection.
Skin scratch: the superficial layer of the epidermis removed using a scalpel.

Corresponding author: Carlow, C.K.S. (carlow@neb.com).
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*These authors contributed equally to this work.

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Table 1. Human filarial parasites: tissue localization, clinical disease, and vectors

Location of adults	Parasite	Clinical symptoms and disease	Location of mf	Arthropod vectors	Refs
Lymphatics	*Brugia malayi*	Fever, lymphoedema, chyluria, elephantiasis	Blood	Mosquitoes: *Anopheles, Mansonia*	[4,5]
	Brugia timori	Fever, lymphoedema, chyluria, elephantiasis	Blood	Mosquitoes: *Anopheles*	[4,5]
	Wuchereria bancrofti	Hydrocele as well as fever, lymphoedema, chyluria, elephantiasis	Blood	Mosquitoes: *Culex, Anopheles, Aedes*	[4,5]
Subcutaneous tissue	*Loa loa*	Migration of adults through subconjunctiva of the eye; dermatitis, itchy skin	Blood	Tabanid flies: *Chrysops spp.*	[2]
	Onchocerca volvulus	Blindness caused by dead and dying mf in eyes; skin nodules, itchy skin	Skin	Blackflies: *Simulium spp.*	[4]
	Mansonella ozzardi	Usually asymptomatic but general malaise possible	Blood	Biting midges: *Culicoides spp.*; blackflies: *Simulium spp.*	[3]
	Mansonella streptocerca	Dermatitis, itchy skin	Skin and blood	Biting midges: *Culicoides spp.*	[3]
Body cavity	*Mansonella perstans*	Generally asymptomatic	Blood	Biting midges: *Culicoides spp.*	[3]

[6]. The main goal of mass drug administration (MDA) campaigns is to interrupt disease transmission by reducing the prevalence of mf [7]. In the absence of an adulticide, it is recommended that MDA should be continued for 10–15 years (http://apps.who.int/iris/handle/10665/66889). MDA programs have now progressed for several years in many areas, and careful monitoring of infection levels in human populations, as well as in vectors, is necessary to evaluate their success, certify elimination, and guide the decision to stop MDA. These activities and overall management of such control programs are most efficiently performed with accurate diagnostic tools suitable for field use. Highly-sensitive methods are required to detect low mf densities in skin or blood, which is often the case when MDA programs are underway [8,9], as well as occult and amicrofilaremic infections [10]. Stringent specificity is required to differentiate the closely related filarial parasites, especially in the equatorial rain forest areas of central and western Africa where loiasis is endemic, because administration of ivermectin can lead to encephalopathy in patients coinfected with *L. loa*. This problem has prevented the implementation of MDA programs and severely impeded control efforts in many areas [11,12].

The current toolbox for filariasis diagnosis and surveillance is limited because many of the available tools suffer from lack of sensitivity and specificity [13] and/or are cost-prohibitive. We summarize the methods in use, most of which have not changed much in the past 20 years, and discuss the prospects for developing new tools based on nucleic acid detection. The rapidly-growing availability of genome sequences from filarial parasites now provides us with unprecedented access to a pool of new potential biomarkers which can be used to develop new molecular diagnostic (MDx) tools for the detection of filarial parasites in both human and vector populations. We briefly describe recent developments in nucleic acid amplification and detection, with a focus on emerging technologies that are field-deployable or suitable for low-resource settings.

Parasitological diagnosis

Filarial diagnosis traditionally centers on morphology of mf using light microscopy and various histochemical stains to determine the presence or absence of a sheath, and the pattern of nuclei in the tail. While this is a valuable technique, morphological interpretation requires substantial expertise and can be subjective. *L. loa* infections can occasionally be diagnosed based on observing the migration of adult worms through the subconjunctiva of the eye [11]. Parasitological diagnosis of *O. volvulus* infection in humans relies on the presence of palpable nodules (onchocercomata) containing adult worms and/or detection of mf in skin snips. However, *M. streptocerca* mf also reside in the skin and can present a diagnostic challenge. In addition, the skin snip test is not a popular diagnostic procedure and requires that the biopsy be removed from areas of presumed maximum mf density, which varies depending on geographic location. In patients with suspected low mf burden, a 'Mazzotti test', following administration of a low dose of diethylcarbamazine (DEC), can be performed. This unpopular option causes mf death, provoking intense itching and erythema in the patient [13].

For detection of mf in blood (*W. bancrofti*, *Brugia* spp., *Mansonella* spp. and *L. loa*), sample collection should coincide with peak levels of microfilaremia. The nocturnally periodic LF (*W. bancrofti* and *B. malayi*) require sampling to be carried out between the hours of 10:00 pm and 2:00 am, which is both labor-intensive and impractical in some areas. A provocative DEC dose during the daytime may also be used to encourage mf to enter the peripheral circulation [13]. Unfortunately this technique will not detect pre-patent or amicrofilaremic infections, the latter of which tend to be common in *L. loa*-infected patients [14]. Because mf prevalence decreases through MDA, screening blood pools has become a necessary and cost-effective procedure [15]. However, this method is more likely to produce false-negative results in low mf carriers that may be infectious to competent mosquito vectors. Despite these limitations, microscopic detection is still a