

Diagnostic tools for filariasis elimination programs

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The ambitious and exciting Global Programme to Eliminate Lymphatic Filariasis (GPELF) is largely based on a strategy of mass drug administration (MDA) of repeated rounds of antifilarial medications to endemic populations around the world. Diagnostic tools are important to GPELF because they affect decisions regarding where to distribute MDA, how to measure its effects, how to define targets and endpoints for stopping MDA, and how to monitor populations for possible resurgence of filariasis transmission following suspension of MDA. This article reviews available diagnostic tests for filariasis and their potential use as tools for different phases of filariasis elimination programs.

Filariasis elimination and diagnostic tools

Lymphatic filariasis (LF) is caused by the nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* and is transmitted by mosquitoes. It is an important cause of physical and social disability that affects over 100 million people in 83 countries in the developing world. Traditional LF control programs were based largely on selective treatment of infected individuals detected by mass screening of blood smears (collected at night) for microfilariae (Mf). Advances in treatment and diagnostics for filariasis led to a paradigm shift in the 1990s that postulated that it might be feasible to eliminate LF. This would be done using selective diagnosis to identify endemic areas followed by repeated cycles of mass drug administration (MDA) to reduce both infection prevalence and transmission rates to levels below those required for sustained transmission [1–3]. The World Health Assembly endorsed this idea in 1997 with resolution WHA50.29, which called for elimination of LF as a public health problem. A Global Programme to Eliminate Lymphatic Filariasis (GPELF) based on MDA was initiated in a small number of countries in 2000 [2] and rapidly expanded to include 42 countries and ~381 million treatments in 2005 [4]. With a target population of 1.3 billion people, GPELF is the largest infectious disease intervention based on MDA initiated to date.

Diagnostic tools are important to GPELF because they affect decisions regarding where to distribute MDA, how to measure its effects, how to define targets and endpoints for stopping MDA and how to monitor populations for

resurgence of LF transmission following suspension of MDA [5]. In the not-so-distant past, diagnostic tools for LF were limited to clinical examination, detection of Mf and detection of antibodies to native-antigen preparations. These tests are inadequate for answering basic questions that are crucial to programs to eliminate lymphatic filariasis (PELFs); however, there have been important advances in filarial diagnostics in recent years. This article reviews current diagnostic tools and their use in PELFs, and highlights the different diagnostic tools and approaches that are needed for different PELF phases (Box 1; Table 1).

Phase 1: mapping and planning PELFs

The first phase of a PELF requires a sensitive, specific and convenient method for detecting LF endemicity that can be used to map endemic areas for inclusion in the program. Overdiagnosis that misclassifies nonendemic areas can greatly increase program expenses and decrease chances for success. Underdiagnosis and exclusion of endemic areas is also not acceptable.

There are several viable diagnostic options for Phase 1 (Box 1). These include: Mf testing, filarial-antigen tests, antibody detection and detection of parasite DNA by PCR in pooled mosquitoes [molecular xenomonitoring (MX)] or in pooled human blood samples. Some programs have used traditional Mf testing (usually with thick blood smears collected at night) in spot surveys to identify endemic areas. This method is insensitive for active infections; it misses people with low Mf counts and those with amicrofilaricemic infections, who have the potential to contribute to future transmission. Blood collection at night for Mf detection is also impractical in some endemic areas. Thus, exclusive reliance on Mf testing could lead to underdiagnosis and exclusion of areas with active LF transmission from PELFs. It is tempting for program managers to focus on the potential advantages of Mf testing, namely that it is specific, inexpensive and requires little infrastructure. However, Mf testing might not have these desirable features in practice because proper sampling of populations, preparation of smears, staining and microscopy are labor intensive. Money saved by reliance on technically poor Mf testing to classify implementation units in Phase 1 of a PELF will be lost, with interest, later in the program.

Available filarial antigen tests detect adult *W. bancrofti* antigens in human blood [6–8]. They do not detect antigens

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Box 1. Diagnostic options for use in PELFs

- Disease prevalence, in the form of hydrocoele, lymphoedema or elephantiasis, is not a sensitive indicator of changes in infection or transmission rates following MDA.
- Mf detection provides data on infection prevalence, parasite density and community Mf load, all of which should decrease following MDA. Proper sampling of populations, preparation of blood smears, staining and microscopy are labor intensive [29].
- Filariasis antigen tests detect antigens released by adult *Wuchereria bancrofti* worms in human blood [7,8,11,30]. Antigen testing by ICT card test (Binax, Inc.: <http://www.binax.com>) or ELISA (TropBio, PTY: <http://www.tropbio.com.au>) is more sensitive than Mf detection and they can use blood collected during the day or night. Antigen levels are related to the number of adult filarial worms in the host [31,32]. The main limitations of commercially available antigen tests are cost, inconsistent availability and their failure to detect *Brugia* infections. Also, antigen testing (as a single test modality) is not good for monitoring progress in the first years of PELFs because many infected people remain antigen-positive for years after treatment, even if they achieve sustained clearance of microfilaremia [33–35].
- The classical method for detection of filarial parasites in mosquitoes uses dissection with microscopy to assess infection (filarial larvae present) and infectivity (infective larvae present) rates over time. Dissection becomes impractical in areas with low mosquito infection rates following MDA. MX detects filarial DNA in mosquitoes by PCR [21]. MX is much more sensitive than dissection for detecting filarial parasites (or parasite remnants) in mosquitoes, but PCR tests cannot tell whether mosquitoes are infective (contain filarial infective larvae). PCR can also be used to detect filarial DNA in pooled human blood samples [36]. This test requires blood samples taken at night in areas with nocturnally periodic Mf. This approach has not been used in population-based field studies to date.
- Antibody monitoring: early antibody diagnostic tests for LF based on detection of IgG antibodies to native parasite antigens were plagued by poor specificity. Newer assays have achieved excellent specificity by detecting IgG4 antibodies to recombinant filarial antigens such as Bm14 and BmR1 [14,15,17]. The Bm14 antibody test is sensitive for infection with (or heavy exposure to) *Brugia malayi* and *Wuchereria bancrofti*, whereas the BmR1 antibody test is sensitive for *Brugia* infections only. Antibody prevalence rates tend to be higher than antigen or Mf rates in young children in LF-endemic areas. Recent studies have shown that antibody rates in young children decrease rapidly in the years following initiation of effective MDA [20].

Urinary antibody tests might also be useful for identifying LF-endemic areas and for assessing exposure of children to filarial infection. Japanese researchers have developed an ELISA with native *Brugia pahangi* adult-worm antigens to detect antifilarial IgG4 antibodies in human urine, and their publications report that this test is a sensitive indicator for infection or exposure to *W. bancrofti* infection [37,38]. They have not yet reported data on the performance of this test with urine samples from areas with brugian filariasis. One attraction of this approach is that it avoids the need for blood samples. However, additional work is needed to verify the specificity of this test; a similar ELISA for IgG4 antibodies to native *B. pahangi* adult antigens in serum had specificity problems with sera from patients with strongyloidiasis [39]. Antibody tests for *W. bancrofti* infection or exposure detect cross-reacting antibodies in sera from people with loiasis and onchocerciasis. This limits the value of antibody testing in areas where LF and other filarial infections are co-endemic (especially sub-Saharan Africa).

The thoughtful use of these tools might be more important than their intrinsic properties. The Japanese PELF succeeded in the 1960s with Mf testing alone as an assessment tool [29].

in blood from patients with other filarial infections (e.g. *Brugia*, *Onchocerca* or *Loa*). Antigen testing is more sensitive than Mf detection and can use blood collected during the day or night. Filarial antigen tests were originally developed as microplate enzyme-linked immunosorbent assays (ELISAs) [9,10]. A rapid-format, immunochromatography (ICT) filariasis card test was introduced in 1997 [11] but took a few years to gain acceptance. However, by 2000 the ICT test was recommended by international authorities as the diagnostic method of choice for mapping the distribution of bancroftian filariasis, and it has been widely used around the world for this purpose in PELFs [12,13]. It is quick (10 minutes), minimally invasive (100 µl blood from a finger prick) and easy to perform. These features freed filarial antigen testing from the confines of research laboratories and enabled its use in field sites around the world. Antibody detection and DNA testing of pooled mosquitoes or human blood samples could also be used to identify areas that are endemic for bancroftian filariasis. However, these tools have not been widely employed for this purpose to date because rapid-format antigen testing is more convenient than available methods for antibody or DNA detection.

Options for mapping areas with brugian filariasis

Despite the limitations of Mf testing listed earlier, the lack of a sensitive antigen test for *Brugia* infections makes Mf testing a strong choice for identifying *Brugia*-endemic areas. Parasite DNA detection (either in mosquitoes or in human blood samples) and antibody tests (based on antigens such as BmR1 or Bm14) could also be used for this purpose [14–16]. Antibody test kits (a dip-stick and a cassette test from Malaysian Bio-Diagnostics: <http://www.mbdr.net>) based on recombinant antigen BmR1 have recently become commercially available. These tests are sensitive for both *B. malayi* and *B. timori* infection and/or exposure [17,18]. Antibody tests have not yet been rigorously validated as tools for mapping the distribution of LF for PELFs. However, there is pressure from *Brugia*-endemic countries to use antibody testing as a mapping tool for PELFs. As an interim measure, the Technical Advisory Group to GPELF recently approved a plan whereby areas with antibody prevalence rates >10% are considered to be endemic and eligible for MDA (i.e. assumed to have Mf prevalence >1%) and areas with antibody prevalence <1% are assumed to have Mf rates <1% [19]. Areas with antibody rates between 1% and 10% require Mf testing to verify endemicity levels.

Phase 2: monitoring progress during a PELF

Interim assessments are essential to demonstrate progress, to identify problems that can be solved while funding is still available and for securing resources needed to complete a program. Requirements for tools for this purpose are somewhat different from those used for mapping endemicity. Consider Mf testing: microfilaremia prevalence rates and community Mf loads typically decrease within months after initiation of MDA programs. Although this is a positive change and evidence of good MDA coverage, it can also be misleading: decreases in Mf prevalence can be transient if the treatment regimens employed are not highly

Table 1. Recommended diagnostic tools for different phases of a filariasis elimination program^a

	Phase 1 Mapping	Phase 2 Impact of MDA	Phase 3 Endpoints	Phase 4 Post-MDA Surveillance
Primary tools	CFA, Mf	CFA, Mf	Mf, CFA ^b	AB ^c , MX
Secondary tools	AB, MX, Blood PCR	AB, MX, Blood PCR	AB, MX	None

^aAbbreviations: AB, antibody testing of sentinel populations, especially children; Blood PCR, detection of filarial DNA in human blood; CFA, circulating filarial antigen tests; Mf, microfilaria testing; MX, molecular xenodiagnosis (detection of parasite DNA by PCR in mosquitoes).

^bCurrent World Health Organization guidelines stipulate endpoints based on Mf and CFA testing. DNA testing of pooled blood samples could replace traditional Mf tests in the future. Financial and technical constraints limit the use of AB and MX in many endemic areas at this time. AB and MX should be included when feasible (see Phase 3, main text).

^cAB might be especially useful for mapping *Brugia* endemicity given the lack of a sensitive antigen test for this infection.

macrofilaricidal. Therefore, although Mf testing will be used in many areas for historical or practical reasons, it is not recommended as a stand-alone tool for monitoring PELFs.

What about antigen testing? Although this is useful for mapping endemicity, caution must be used if this tool is used for interim assessments of PELFs, especially early on. This is because filarial antigenemia rates decrease more slowly than Mf rates after MDA. Thus, antigen testing will tend to underestimate effects of MDA on microfilaremia and transmission. However, antigen prevalence rates do decrease substantially after several rounds of effective MDA. A study in Egypt documented a decrease in antigen prevalence from a baseline rate of 19% to 4.8% after five annual rounds of MDA [20]. Mf prevalence in the same area decreased from 11.5% to 1.2% by membrane filtration assessment (0.3% by 50 μ l thick smear). Antigen prevalence in areas with lower baseline endemicity rates fell to <1%. If low residual antigen prevalence rates can be achieved, the risk of resurgent LF should also be low following suspension of MDA (assuming no reintroduction because of migration).

Based on these considerations, Mf and antigen testing are considered to be complementary, and a combination of antigen and Mf monitoring is preferable to either test used alone. Studies in Egypt have used antigen testing as the primary monitoring tool, with Mf testing limited to people with positive antigen tests. It is, therefore, recommended that PELFs conduct antigen testing of sentinel populations to establish baseline rates before the first round of MDA. This should be repeated before the third round of MDA and no more frequently than every two years thereafter.

Tools for monitoring transmission

Recent data from Egypt [20] and unpublished results from other geographical areas show the value of antibody testing and MX as tools for interim assessment of PELFs. Antibody tests are used to detect infections or heavy exposure to filarial parasites [15] and MX detects filarial DNA in mosquitoes by PCR [21]. These tests are sensitive to changes that occur during a successful PELF that indicate that the program is on course. Although neither test directly measures transmission, they do provide information on changing rates of LF transmission and the potential for transmission, respectively. However, these tests are now at the stage where antigen testing was before the ICT card test appeared in 1997 – interesting research tools not yet ready for broad program use. Antibody testing in *W. bancrofti* endemic areas will not be widely practiced until reliable commercial kits become available. MX is much more sensitive than dissection for detecting filarial parasites (or parasite remnants) in mosquitoes.

Unfortunately, MX is beyond the capabilities of many laboratories in filariasis-endemic countries and no national PELF uses this method for monitoring at this time. However, recent technical advances have the potential to change MX from a research tool to a practical monitoring tool that can be used by PELFs around the world. These include the use of traps for more efficient collection of mosquitoes (e.g. gravid traps for *Culex*, light traps for *Anopheles*), improved methods for isolating DNA from mosquito pools, software for calculating mosquito infection rates from qualitative PCR results [22,23] and real-time PCR for specific amplification of parasite DNA and detection of amplified DNA [24,25]. More research is needed to determine the relative value of antibody and MX testing as monitoring tools: each has advantages, and the two approaches are complementary. Antibody monitoring of sentinel populations provides information on the cumulative lifetime exposure of the sampled cohort to filarial infection. This method requires collection of finger-prick blood from a sentinel population such as first-year primary school children [20]. MX is based on the ability of mosquitoes to collect human blood, and these ‘flying syringes’ do this for a living. MX provides information on the point prevalence of filarial parasites in mosquitoes in the area sampled. In practice, most parasite DNA detected by MX in mosquitoes is from pre-infective stages. Therefore, MX should be thought of as a means of efficiently sampling endemic populations for the presence of microfilariae. It is not a measure of infectivity or current rates of parasite transmission.

Phase 3: endpoints for PELFs

More information is needed on how to use antigen, antibody and MX tests to inform decisions on when it is safe to discontinue MDA because there is no consensus on this issue at this time. The authors favor an evidence-based approach to this question rather than adoption of arbitrary targets. We do not agree with current WHO guidelines that fail implementation units (requiring additional rounds of MDA) if one in 3000 children born after the initiation of MDA has a positive antigen test [5]. We believe this target is well beyond what is needed to eliminate LF (or to reduce transmission to unsustainable levels). More data are needed to determine residual Mf prevalence rates that correspond to interruption of transmission in different situations (or reduction of incidence rates to well below rates of spontaneous clearance of filarial infections). The Mf threshold rate of 1% for sustained transmission reported from China [26] might not be valid everywhere, but there is no evidence that a Mf prevalence rate below 0.5% can sustain transmission in non-*Aedes* transmission

areas. If a target Mf prevalence rate of $<0.5\%$ could be accepted (as suggested for areas with transmission by *Culex* mosquitoes) [27], this would be much easier to achieve and document than an Mf or antigenemia rate of $<0.1\%$.

It is difficult to demonstrate the absence of filarial infection or transmission because assessment tools are not perfect and financial constraints place limits on the number of samples that can be collected and tested. Therefore, it is better to use statistical criteria for targets. For example, sample sizes should be calculated to provide 95% certainty that the true rate is less than the target rate, with β or type-2 error (the likelihood that a measured rate is falsely above the target rate because of skewed sampling) $<20\%$. Target rates do not need to be zero: they should be rates that are below those needed for sustained transmission of LF. A small number of new cases can be accepted if the incidence rate is well below the attrition rate for LF infections.

Returning to targets, we favor the idea of developing provisional targets based on data from population-based studies of MDA and then testing these targets with field studies. For example, studies in Egypt have suggested the following provisional targets for treated populations after at least five years of effective MDA: $<2\%$ for antigenemia (which corresponds to a MF prevalence of $<0.5\%$) [20], $<2\%$ for antibody prevalence in first-year primary school children and $<0.25\%$ for mosquito infection rates by MX. A study is being initiated that will test whether communities that have achieved these targets have reduced transmission below sustainable levels. Different targets might be needed in different areas because of differences in vector species, baseline infection rates, seasonal transmission patterns, biting rates and the local choice of drug(s) used for MDA.

Phase 4: post-MDA surveillance and early detection of resurgence

We favor the use of antibody testing and MX as surveillance tools for early detection of resurgent transmission. Large samples are needed to show statistically significant increases in these measures when baseline rates post-cessation of MDA are low. There is no consensus at this time on how these or other tests should be used for post-MDA surveillance. Research is required to provide data on this important question. Surveillance studies in Egypt will repeat assessments in sentinel sites every other year; these assessments are powered to detect increases in mosquito infection rates or in antibody rates in first-year primary school children.

Advanced data analysis and modeling

Two recent articles have reviewed the potential value of mathematical modeling as a tool for PELFs [27,28]. Research projects and PELFs around the world are generating reams of data from specific villages, regions and countries. Mathematical analysis and modeling efforts should be expanded to identify patterns in the data so that the field can move from the specific to the general. W. Stolk (personal communication) has commented on this situation as follows: 'While field studies are useful, there are few

good ones and even the best of them are limited in scale and time by budget constraints. Moreover, it is not possible to test every intervention in every situation. Modeling can help overcome these limitations by intelligently predicting outcomes of studies that have not been conducted.'

Modeling has the potential to be helpful for people in the real world who are responsible for making the tough decisions about PELFs. Managers need practical guidance on criteria for including areas in MDA programs, on when it is reasonable to stop MDA, on adding complementary measures such as vector control, on how to look for early evidence of LF re-emergence and on options for managing this situation if it happens. Modeling studies depend on the quality of the data available for analysis and this has been a limitation in some prior modeling efforts for LF. Existing models should now be refined by incorporating new types of data (antigenemia, antibody and mosquito infection rates) to analyze relationships between infection and transmission parameters. In addition, more high-quality field data are needed to 'ground-truth' these models. Although it might not be possible to do this for every combination of vector, parasite and environment, modeling should be done with field data from several of the major LF transmission zones. Standardized data collection protocols would help to ensure that comparable data are collected in different areas.

Concluding remarks

GPELF is one of the most ambitious and exciting intervention programs for a neglected tropical disease to date. This review has emphasized how recent advances have increased diagnostic options for filariasis and that different diagnostic tools might be needed for management of different phases of PELFs. More work is needed to fill important gaps in our knowledge regarding the optimal use of these tools (Box 2).

Box 2. Diagnostic tools for GPELF: research priorities

- Improve reliability and availability of rapid-format filarial antigen tests (preferably at a reduced cost).
- Field studies are needed to validate the use of parasite DNA detection with pooled human blood samples and PoolScreen data analysis as a means of estimating Mf prevalence rates in areas with brugian and bancroftian filariasis.
- Develop and validate improved methods for assessing filariasis transmission that are practical for use by PELFs.
- Develop and test improved protocols for post-MDA surveillance for early detection of persistent or resurgent LF transmission.
- Determine the importance of persistent parasite antigenemia in people who are Mf-negative following treatment with either DEC with albendazole, or albendazole with ivermectin. How often do they develop recurrent microfilaremia in the years following treatment?
- Develop a specific filariasis antibody test for sub-Saharan Africa that is not cross-reactive with sera from people with loiasis or onchocerciasis.
- Develop molecular assays for specific detection of infective filarial larvae in mosquito pools.
- Find ways to make MX more practical for use by PELFs. This includes technical improvements in mosquito sampling protocols, DNA isolation and PCR testing, and operational advances such as establishing a network of regional reference laboratories for MX testing.

Disclosure statement

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