2. RESEARCH DIRECTLY LINKED WITH GPELF ACTIVITIES (OPERATIONAL RESEARCH)

2.1 ESSENTIAL TOOLS—DIAGNOSTICS

Patrick J. Lammie**

Summary of Prioritized Research Needs

1) Develop effective, practical strategy for defining in field settings the areas and individuals with levels of *L. loa* microfilaremia so high as to be dangerous if MDA for concurrent LF were to be initiated (novel tool; novel approach with available tools).

2) Define the comparative accuracy of available diagnostics (antigen, antibody, DNA) and strategies for monitoring the progress of LF elimination programs and for deciding both when to stop MDAs and how to initiate surveillance to detect potential recrudescence:
   a) involves determining limits of sensitivity and specificity of available tests,
   b) requires longitudinal studies using all diagnostic tools concurrently and in both high- and low-prevalence areas where LF elimination programs are underway,

3) Take advantage of new technologies to improve user-friendliness and efficiency of the LF diagnostics currently available (e.g., isothermal polymerase chain reaction [PCR], multiplex antibody or PCR kits, use of oral fluids or urine for diagnostic tests).

4) Validate sampling strategies for testing both vector and human populations for LF infection or exposure to infection.

2.1.1 Overview

Principal challenges for LF diagnostics.

Diagnostic tools are essential at each step of the GPELF, initially for defining areas in need of MDA through mapping activities, then for monitoring the progress of programs following implementation of MDA, and finally for verifying the absence of infection both in areas where MDA has been conducted and in settings where LF was present historically. Existing tools are thought to be adequate for these purposes, but validation, refinement, and additional practical experience with the available assays are clearly needed.

As LF programs approach their planned end points (five or more years with greater than 80% MDA coverage), it will be necessary to determine whether transmission has been interrupted and whether MDA can be stopped. Parasitologic assessment, whether by microfilaremia or antigenemia, will require testing thousands of persons to demonstrate that infection levels are below 0.1%, the level targeted by WHO. Current test strategies represent a best guess about approaches likely to be effective based on detection of microfilaria or use of the immunochromatographic test (ICT) for antigenemia. To introduce use of entomologic tools or antibody testing for this type of decision-making will require an understanding of how these new tests perform. Intensive data collection, in the context of ongoing programs, will be needed to determine how these different tests for antigen, antibody, and vector infection correlate and compare with microfilariaemia as a programmatic endpoint. Age- and sex-specific longitudinal data collections (in a variety of epidemiologic settings) are the key to developing the ability to recognize with confidence when transmission has been interrupted.

Preliminary guidelines for verifying the absence of LF transmission have been set by WHO (www.filariasis.org). Testing the effectiveness of the available diagnostic assays in the application of these guidelines also should be a priority. Since test specificity is of greatest concern for this issue, algorithms used to confirm suspected infection or exposure must be developed and validated. In addition, population movement from endemic to nonendemic regions carries with it the risk of possible introduction of LF transmission. Surveillance methods to address these situations, based on diagnostic and serologic methods, should be evaluated.

**LF diagnostics today.**

To date, LF infections have been diagnosed principally by direct demonstration of the parasite, initially through microscopic detection of microfilariae (mf) in the blood of infected persons and more recently by detection of parasite antigen or DNA.

While microscopic detection of parasites will continue to play an important role for demonstrating the impact of community-wide interventions, measurement of microfilaremia is not an ideal tool for program monitoring or surveillance, both because of 1) the need to examine nocturnal blood to find the parasites in most areas of the world and 2) the relative insensitivity of the commonly used methods for mf detection.

For *Wuchereria bancrofti*, assessment of antigenemia offers the convenience of any-time-of-day testing and greater sensitivity than testing for mf; however, current tests are specific for *W. bancrofti* and do not detect *Brugia* (*B. malayi* or *B. timori*) infections. Polymerase chain reaction–based methods can be used to detect infection in humans and to monitor filarial infection in mosquitoes with exquisite specificity and a sensitivity greater than that of direct microscopic detection of mf (or, in the mosquito, other larval stages), but current tests require a relatively sophisticated laboratory infrastructure. Also, since the technique identifies DNA still in the mf in blood samples from humans (i.e., not DNA freely circulating in the blood), its diagnostic usefulness is still constrained by microfilarial periodicity.

Antibody assays, though not detecting the parasite directly, in principle provide sensitive and relatively inexpensive tools to measure filarial exposure; however, the application of an-
2.1.2 Research Needs

**Improvements in existing tools.**

**Antigen detection.**

Adult *W. bancrofti* release antigens that can be detected in human blood, plasma or serum by immunoassay. Unlike mf, circulating antigen can be detected with blood collected during the day or night. There are currently two antigen detection tests on the market. An enzyme-linked immunosorbent assay (ELISA) (which detects Og4C3 antigen and is produced by TropBio, Townsville, Queensland, Australia) is highly sensitive and specific; however, this test requires a back-up laboratory infrastructure and is mainly used in research projects.5 The other antigen test (the ICT filariasis test, which detects AD12 antigen and is produced by Binax, Portland, ME) is a rapid-format ICT.3 It can directly test blood, serum or plasma in the field and provides a result in 10 minutes. This test is widely used around the world to identify or map endemic areas for inclusion in MDA programs.1 This antigen card test has provided much better information on the distribution of filariasis than traditional testing of night blood for mf. Indeed, card testing has identified many highly endemic areas that were not previously recognized as being endemic for LF.4

One important limitation of antigen testing is that it currently does not detect *Brugia* infections (which account for ~10% of the world’s burden of LF). Another is that antigen test results often remain positive after treatment with DEC/albendazole or DEC/ivermectin.5,6 This is probably because these drug regimens are not completely effective in killing adult worms, but it is also possible that even when all adult worms are killed, antigen clearance from the blood takes some (still undefined) period of time. Furthermore, when antigen prevalence rates are low in young children, as they are in some LF-endemic areas, measuring incidence rather than infection prevalence is more valuable for monitoring changes in transmission of filariasis following implementation of MDA.

The ICT is now an essential tool for mapping the prevalence of filariasis. Should it fail to continue working for any reason or become unavailable for commercial reasons, the operation of the GPELF would be seriously compromised. The ELISA, which is slightly more sensitive than the ICT and provides a quantitative result, is widely used in filariasis research. Because both tests recognize the same circulating antigen, a strategic and sensible research goal would be

- **the development of a complementary, alternative antigen-detection test.**

Neither the ICT nor the Og4C3 ELISA can detect *Brugia* antigens. Consequently, for defining areas where *B. malayi* or *B. timori* are endemic and for defining changes in infection post treatment, antigen detection is not yet possible. Therefore, an important diagnostic initiative would be

- **the development of an antigen assay that could be used in *Brugia*-endemic areas.**

**DNA Detection.**

*Filarial* parasites contain repeated DNA sequences that can be amplified and detected in humans or vectors by PCR. Currently, assays exist for screening blood samples and vectors for *W. bancrofti*, *B. malayi*, *B. pahangi*, *B. timori*, *L. loa*, and *Mansonella streptocerca*.7–11 The sensitivity and specificity of the PCR have also made these tests particularly useful for validating the results of conventional parasitologic tests where doubts about species identification arise. Although PCR assays are widely used, their utility could be enhanced by

- **improving the availability of standard kits for blood-sample collection, DNA isolation, PCR amplification, and DNA product detection.** For use in the widest range of field settings, kits that permit isothermal amplification and visual detection of PCR products without instrumentation would be ideal.

- **developing a multiplex PCR that could be used to detect and differentiate *Brugia* spp. and *Wuchereria* from other filarial parasites.** If such an assay included *Plasmodium* or other pathogens, this would provide additional information that could increase the value of the PCR tests and improve linkages to other health programs.

**Mosquitoes.**

The PCR is much more sensitive than traditional dissection and microscopy for detecting filarial parasites in mosquitoes.12 Studies in a number of settings have shown that the percentage of PCR-positive mosquito pools decreased dramatically following MDA.13,14 In addition, standardized protocols for detecting DNA in vectors have been developed through multicenter collaborations.12 However, a number of practical challenges must be overcome before the PCR can be used with entomologic techniques for xenomonitoring the program effects on LF transmission; particularly,

- **since different vector species are responsible for transmission of LF around the world, appropriate techniques for trapping mosquitoes to sample the mosquito populations accurately must be developed,**

- **sampling protocols need to be validated to define how or if these protocols should change as infection prevalence decreases following MDA.**

**Antibody detection.**

In principle, antifilarial antibody responses can serve as very sensitive markers of filarial exposure and transmission, providing evidence of infection in an individual long before the development of antigenemia or microfilaremia, since antibody responses may develop within weeks to months following exposure to infective larvae. Thus, assays for antifilarial antibodies should be able to be used for program monitoring following initiation of filariasis elimination programs. Furthermore, compared with parasitologic or entomologic methods that estimate transmission at a single point in time, antibody responses represent a cumulative measure of the experience of infection, an important advantage for a monitoring or surveillance tool.
Native antigens isolated from filarial worms frequently cross-react with antigens from other nematode parasites. However, two recombinant-antigen (Bm14 and Bm-R1)–based antibody tests have been shown to be sensitive and specific for LF infection/exposure. The Bm14 antigen is equally sensitive for both Wuchereria and Brugia infection or exposure. This antigen has some cross-reactivity with sera from patients with other filarial infections (loiasis and onchocerciasis), but not with sera from people with non-filarial nematode infections. Field studies in Egypt showed that prevalence rates of antibody to Bm14 prior to initiation of MDA were much higher than antigen or mf prevalence rates in young children. In addition, follow-up studies have shown that antibody prevalence rates in children decreased rapidly in the years following implementation of MDA.

The Bm-R1 antigen performs well in antibody tests for B. malayi infection/exposure (sensitivity > 95% and specificity = 100%), but it has limited sensitivity for W. bancrofti infection. The Bm-R1 antibody test detects IgG4 antibodies (generally, though not always, associated with active infec-

tion) and is available commercially in ELISA and rapid-format versions (Malaysian Diagnostic Research Sdn. Bhd., Kuala Lumpur, Malaysia). It is currently being evaluated as a potential tool for mapping and monitoring program activities in Brugia-endemic areas. Additional studies are needed to define the relationship between its seroprevalence and either microfilaremia or antigen prevalence.

Even as antibody assays are developed and validated, especially for use as potential surveillance tools to detect exposure to infection, there are a number of important research questions that should be answered. Most current assays have focused on detecting antifilarial IgG4 because of the greater specificity of the IgG4 (vis a vis IgG1) response or because of the lack of an IgG4 response to the fusion partner used to express the recombinant antigen. In principle, IgG1 responses should develop sooner after exposure than IgG4 responses; however, it is not clear whether this difference is of practical significance in the field. Therefore, it is important

- to determine whether IgG1-based assays can be more effective diagnostics than those currently based on IgG4.

Since experience is lacking in the practical use of antibody assays in the context of LF programs, it is also necessary

- to assess whether children provide the best sentinel population; is there a preferred sampling strategy?
- to determine how the incidence of antibody responsiveness and the prevalence of antibody responses change in the aftermath of MDAs.

New diagnostic tools.

Diagnostic tests for L. loa infection.

The occurrence of serious complications from ivermectin administration, including fatal encephalopathy, in areas co-endemic for onchocerciasis and L. loa infection presents a major obstacle to LF elimination in Africa since the distribution of LF overlaps that of loiasis. Indeed, the extension of the GPELF into areas co-endemic for loiasis has been completely halted. Since current data indicates a relationship between high L. loa blood mf levels and the likelihood of an adverse reaction, the decision to extend treatment into a given area should depend not only on whether loiasis is present, but also on whether there is high-intensity Loa microfilaremia or whether individuals with high levels of Loa microfilaremia can be effectively excluded from MDA activities.

Although a number of epidemiologic tools, including geographic information systems and rapid assessment procedures for loiasis (RAPLOA), have proven useful in identifying regions in which L. loa is endemic, difficulties remain in defining those areas where individuals at greatest risk of adverse outcomes (i.e., those with very high levels of microfilaremia) are found. Currently available tools for diagnosing individuals include microscopy, a Loa-specific PCR, and serologic tests. Of these, only microscopy is able to provide a quantitative estimate of the level of microfilaremia, and even this approach is hampered by the periodicity of the L. loa mf, the technical expertise required to distinguish L. loa from Mansonella perstans in the blood, and the time required to prepare, stain, and read the slides. Loa-specific PCR assays and serologic tests using recombinant Loa antigens have been developed, but are neither quantitative nor field-adapted at this time. To address these issues, research is needed at two levels.

- To evaluate currently available technologies (including quantitative thick smears, RAPLOA, and Loa-specific PCR) for their usefulness in identifying regions and individuals at highest risk; this will require epidemiologic studies in areas of low and high prevalence L. loa infection in the presence and absence of co-endemic M. perstans.
- To develop novel diagnostic assays and/or adaptations of currently available assays that can rapidly identify individuals with high intensity Loa microfilaremia in a field setting.

New diagnostic tests for LF.

Although a recent multicenter evaluation identified several promising candidate antigens for serologic assays to monitor LF, none was specific for W. bancrofti. Because LF elimination programs may have limited effect on persons with or exposed to Mansonella, Loa, or Onchocerca volvulus infections, the absence of a specific for assay W. bancrofti will make it difficult to use current antibody tests for program monitoring in areas where these infections are co-endemic with LF. For example, testing young children in Egypt for antifilarial antibody using the Bm14 antigen is useful as a method to detect recent exposure to W. bancrofti; in contrast, an incident antibody response in Nigeria may only represent exposure to other filarial parasites. Therefore, a valuable research goal is

- the development of an antibody test that is specific for W. bancrofti to enhance the usefulness of ‘exposure antibody’ assays for program monitoring in sub-Saharan Africa.

Collection of blood specimens remains, in many places, a significant programmatic challenge. Therefore, to address this practical program concern, attempts should be made to

- adapt the existing serologic assays to work with oral fluids or urine that would provide program managers with other options to achieve complete or adequate sample collection.
2.1.3 References


