

Introduction

Effective monitoring and evaluation is not only necessary during the mass drug administration (MDA) period but important throughout the lifespan of lymphatic filariasis (LF) programs, including after MDA has stopped. Current WHO recommendations for surveillance advise programs to implement activities to detect new foci of transmission through the assessment of microfilaremia, antigenemia or antibodies. There is a critical need for reliable diagnostic tools that can be used to guide programmatic decisions, especially decisions made in the final stages of the program and in areas where co-endemic filarial diseases complicate program activities. Although there are diagnostic tools available to detect microfilariae (Mf), *Wuchereria bancrofti* circulating filarial antigen (CFA), and antifilarial antibodies, antibody responses are detectable earlier than CFA or Mf (Gass et al) and provide the earliest indicator of filarial exposure. Furthermore, antibody responses decline after MDA (Steel et al), potentially offering opportunity to use antibody responses for surveillance.

The objectives of the meeting were:

- **To review existing antibody data;**
- **To determine programmatic use cases using current antibody tools; and**
- **To determine potential programmatic use cases using modified/new antibody tools**

Age Prevalence

In order to determine the utility of antibody tools for LF programs, there is a need to establish age prevalence curves for antigen and antifilarial antibody responses in pre- and post-MDA settings. Characterizing antigen and antibody responses in these settings will help to determine the appropriate age group(s) to monitor and diagnostic tool(s) to use.

Five presentations were given during this session. Presenters and topics were as follows:

1. "Pre-MDA: Gambia, Mozambique" – Kim Won
2. "Post-MDA: Kenya" – Sammy Njenga
3. "Post-MDA: Nigeria" – Greg Noland
4. "Post-MDA: India" – Swaminathan Subramanian
5. "Post-MDA: Philippines, American Samoa, Tanzania" – Kim Won

Key results:

- Pre-MDA/no MDA
 - In a high transmission setting (Mozambique) there was higher prevalence of antibodies to Wb123 and Bm14 than CFA and mf; antibodies to Wb123 and Bm14 were detectable in children <5 years old
 - There was a steep increase in antibody prevalence with increase in age
 - There was a near absence of detectable responses to Wb123 in a population with historic evidence of high LF transmission (Gambia); Bm14 responses were restricted to individuals >50 years old
- Post-MDA

- There was a higher prevalence of antibodies to Wb123 than CFA when measured by ELISA, multiplex bead assay (MBA), LIPS (Kenya, India, American Samoa, Philippines, Tanzania); Wb123 prevalence by rapid diagnostic test (RDT) was similar to CFA prevalence (Nigeria)
- Antibody prevalence appears to increase with age
- In general, Wb123 prevalence is low among children in areas where transmission is suspected to be low; antibody prevalence is higher among children in areas where transmission is suspected to be sustained or increasing

Discussion points and operational research (OR) opportunities:

- Choice of diagnostic platform (i.e. RDT, ELISA, MBA) will impact data interpretation; challenging to compare data because of different diagnostic platforms used; commercially available tests should be used to standardize results
- Lack individual test concordance observed in several settings; is there a need for an antibody test that detects all antigen positive individuals?
- The duration of antibody responses is unclear
- **Cutoff determination of lab-based assays presents a challenge. Further analysis needed to determine best approach for cutoff determination; one possible analysis could be to use signal to noise instead of normalized optical density.**
- **Further investigations are needed to determine the rate at which antibodies decrease over time.**

Change Over Time

Antibody tools represent an important opportunity for LF surveillance, as antifilarial antibodies are an early indicator of exposure and, consequently, are more prevalent in a population than either antigenemia or microfilaremia. The ability to measure a significant decline in either the prevalence or intensity of antifilarial antibodies overtime might lead to improved strategies for post-MDA surveillance.

In this session, the following four presenters shared country-specific data assessing the change in antibodies over time:

6. "Bangladesh" – Gretchen Cooley
7. "Philippines" – Katie Gass
8. "American Samoa" – Colleen Lau
9. "Indonesia" – Peter Fischer

Key Results:

- The period of surveillance and the sampling approach both appear to affect whether it is possible to detect a decline in antibody levels over time
 - A census of the same two sentinel sites in the Philippines, conducted 16 months apart, was able to detect a significant decrease in the quantitative Wb123 response, by ELISA
 - A hospital-based surveillance system in Bangladesh did not detect a significant change over time in either Bm14 or Wb123, both by ELISA, during a 3 year period; however, it

was possible to differentiate between the endemic and non-endemic areas based on the antibody signals detected, using these same data

- Wb123 and Bm14 response (assessed by either ELISA or multiplex) appear to be more sensitive and rise faster than FTS and may be useful for detecting a resurgence of infection or predicting a TAS failure
- BmR1 appears to decline over time in the entire population post-MDA in a manner that is consistent with decreasing levels of mf

Discussion points and OR opportunities:

- Brugia rapid appears to be suitable to measure the effect of MDA in Brugia areas and may be acceptable for use in pre-TAS
- Post-MDA, the distribution of antibody responses shifts to the left, so we are looking for a signal that will be diminishing over time
- **Further investigations are needed to determine the rate at which *Wuchereria bancrofti* antibodies decrease over time across different age groups; sentinel sites may offer the best opportunity for follow-up**

Spatial Distribution

As elimination efforts are successful, any remaining pockets of infection tend to become increasingly focal. Detecting these foci (aka clusters or hotspots) of infection with antigenemia can represent an expensive challenge for programs; like looking for a needle in a haystack. Understanding the spatial distribution of antibody responses may ultimately lead to improved detection of transmission foci, if antibody responses exhibit greater spatial correlation than antigen or mf.

This session had two presenters, sharing published and unpublished data on the spatial distribution of antibodies:

1. "Published data" – Kim Won
2. "American Samoa" – Colleen Lau

Key Results:

- Significant spatial heterogeneity of antibody response may exist, especially in post-MDA settings
- Antifilarial antibody responses appear to cluster over space and these clusters can be identified with current statistical software (e.g. SaTScan)
- Increasing antibody response (measured by ELISA) appears to be associated with an increased odds of living near a CFA positive individual

Discussion points and OR opportunities:

- Optical density values have been shown to have little to no relationship with mf, but the association with antigenemia has not been assessed
- The scale of spatial clustering will have implications on the optimal sampling strategy, the broader the spatial correlation, the fewer data points required
- We should consider using the flight range of the mosquitoes (particularly in *Anopheles* and *Culex* areas) when determining how to follow-up positive results

Transmission Assessment Survey (TAS)

The WHO-recommended transmission assessment survey (TAS) was designed as a decision-making tool to determine when transmission of LF is presumed to have reached a level low enough that it cannot be sustained even in the absence of MDA. To date, 1,093 TAS have been conducted globally with greater than 90% pass rate (WER 2017). However, the TAS is not powered to detect change in antigen prevalence over time and therefore may not be the best approach for the post-MDA surveillance period. Inclusion of complementary indicators in TAS 2 and TAS 3 may provide useful information on the status of LF transmission after stopping MDA. Monitoring filarial exposure through the assessment of antibody responses may provide a useful tool for detecting potential recrudescence.

Three presentations were given during this session. The presenters and topics were as follows:

1. "Haiti TAS (2015)" – Kim Won
2. "Tanzania" – Katie Gass
3. "Mali" – Tom Nutman

Key Results:

- In Haiti, several evaluation units (EUs) passed TAS 1 by ICT, but antibody responses may have been indicative of future TAS failures
- Antigen positive children identified in school-based TAS may represent LF status in communities
- Antibody responses may provide additional information on TAS outcomes compared to antigen responses alone
- In Mali, prevalence of antibodies to Wb123 was low by Wb123/Ov16 bplex; prevalence was similar by ICT

Discussion points and OR opportunities:

- **Further investigation is needed to determine how to follow up antigen positive cases identified in TAS**
- **Further investigation is needed to determine if clustering of antigen or antibody children in TAS should result in programmatic action**

Specificity

There is a need to provide guidance for LF programmatic activities in *Loa loa* co-endemic areas. Because of the inability to use antigen tools, antibody tools may provide an option to provide clarity.

Three presentations were given during this session. Topics and presenters were as follows:

1. “Cameroon” – Tom Nutman
2. “Cameroon” – Allison Golden
3. “Gabon” – Pat Lammie

Key Results:

- Responses to Wb123 appears to be parasite specific; in a large study in Cameroon, Wb123 responses by Wb123/Ov16 bplex were not influenced by *Loa* or *Mansonella* mf intensity
- In a separate study conducted in a *Loa*-endemic area of Cameroon, there was significant discordance between RDT and ELISA platforms
- Wb123 assays can be used to resolve questions about LF when positive FTS results are observed

Discussion points and OR opportunities:

- Wb123 testing should be population-based and not only of FTS-positive individuals due to the lack of concordance between FTS and Wb123
- Decisions about LF endemicity should not be made based on sentinel site surveys. As recommended by WHO, appropriately designed cluster surveys should be conducted.
- **More investigation is needed to determine whether the discrepancy between the RDT and ELISA in *Loa* settings is due to cross-reactivity**

Test Concordance (see Concordance table included in Annex):

Understanding the concordance of diagnostic tests, particularly for tests measuring the same indicator (e.g. Wb123 RDT vs. Wb123 ELISA), is necessary for interpreting data across diagnostic platforms, setting programmatic thresholds, and determining the appropriate use cases for each available diagnostic.

No presentations were given during this session, instead a table with test concordance (see Annex) was presented and discussed.

Key Results:

- There is little concordance between Wb123 RDT and FTS positivity within individuals in post-MDA settings
- The Wb123 prevalence, assessed by RDT, appears to be consistently less sensitive than Wb123 prevalence assessed by either ELISA or multiplex

Discussion points and OR opportunities:

- Wb123 testing must be population-based and not limited to FTS-positives due to the lack of concordance between FTS and Wb123 post-treatment

- **There is significant discordance between the RDT and ELISA platforms, particularly in *loa loa* settings. More investigation is needed to understand whether some of the discrepancy observed is due to cross-reactivity**

Molecular Xenomonitoring and Human Indicators

Molecular xenomonitoring (MX) could be a potentially useful indicator of human infection and used to evaluate the success of programs. Although MX offers advantages of being non-invasive to humans, it requires entomological expertise and there is still some uncertainty on appropriate sampling strategies. There is a better need to understand the relationship between mosquito and human indicators in order to determine how the tools can best be used in LF programs.

Two presentations were given during this session. The topics and presenters were as follows:

1. “American Samoa” – Colleen Lau
2. “Sri Lanka, Egypt, Papua New Guinea” – Gary Weil

Key Results:

- In American Samoa there was a relationship between positive mosquitoes and Wb123 positivity; no relationship was observed between positive mosquitoes and Bm14 positivity
- MDA has an impact on filarial DNA rates in mosquitoes
- Positive mosquitoes and antibody responses are sensitive measures for detecting low level transmission

Discussion points and OR opportunities:

- MX may not be feasible in all program settings
- **Further investigation is needed to determine the potential role of MX in investigating TAS outcomes (e.g. TAS failure)**

Field Laboratory

High quality data are critical to ensure reliability and comparability of laboratory results for programmatic decision-making. Ideally, standardized protocols, quality control and assurance activities, and training can be implemented across all laboratories.

Four presentations were given during this session. Presenters from the representative laboratories shared information on laboratory capacity and their experiences with the InBios Wb123 ELISA.

1. “Bangladesh” – Sharmin Sultana
2. “India” – Swaminathan Subrimanian
3. “Kenya” – Sammy Njenga
4. “Togo” – Rachel Bronzan

Key Results:

- All labs successfully used the InBios Wb123 ELISA
- Several challenges were identified across the labs:
 - Staff rotation and turnover
 - Storage conditions of samples (-20 °C) difficult due to infrastructure and power cuts
 - Difficulty in organizing the transfer of samples from site of collection to central laboratory for testing
 - Throughput
 - Standardization of results
 - Data management/data interpretation
 - Importation of commercial products

Discussion points and OR opportunities:

- RDT is the preferred format for programmatic use

RDT Modification

Rapid diagnostic tests (RDTs) enable programs to make quick, standardized decisions in the field and are the preferred diagnostic format for programmatic use. The two use cases for a Wb123 RDT are as 1) a mapping tool where loa loa is co-endemic and 2) for post-MDA surveillance. While the recently developed Wb123 RDT (and Wb123/Ov16 bplex) has demonstrated great feasibility in the field and a strong performance in the lab, in its current format, the Wb123 RDT is not a sufficient tool for either use case due to the poor sensitivity of the test. It will be important to learn from the successes and challenges involved in the development of the Wb123 RDT to accelerate the development of new and improved tools.

This session had one presenter, representing the work done by PATH to develop the new Wb123 RDTs:

1. “RDT Modification” – Roger Peck

Key Results:

- It is important to be clear on the specific use cases for new diagnostic tests upfront
- Whether we choose to better understand the performance of the existing tools vs. modify the existing tools vs. develop new tools has major implications for funding and time
- More samples are needed from across the programmatic settings (preMDA, preTAS, TAS, and Post-MDA) in order to better understand the performance of the RDT and determine whether adjustments are needed
- Any change to the current tools will require close partnerships for sharing of samples, product evaluation and field validation

Discussion points and OR opportunities:

- Multiplex is not a programmatic tool
- Issues with cutoff determination, resources required, and interlab standardization make the ELISA similarly impractical for programs
- We need an RDT that is simple and practical for point-of-care use in the field

- The most important use-case for LF antibodies will be as a tool for surveillance
- In its current format, the Wb123 RDT is of limited use because it is no more sensitive than the FTS
- Wb123 may have limited programmatic utility for mapping because the FTS will remain the decision-making diagnostic tool; however, in *loa loa* settings where cross-reactivity is a concern, the Wb123 may help to interpret the FTS results
- If the current Wb123 RDT tool is not sufficient, target product profiles (TPPs) may be needed
- Samples from across the different programmatic use cases need to be shared with the test developer and partnering labs in order to validate the performance of any RDT (new or existing)
- Need to keep in mind that, for surveillance, the population we will be testing will likely be young children, so any antibody tool needs to be able to detect low level infections and the samples used when evaluating and validating the RDT should reflect this
- The Wb123 ELISA is needed as an interim tool for *W. bancrofti* areas until we feel better about the performance of the RDT
- **In parallel to improving the current tools we need to be investing different isotypes and new antigen targets**
- **Head-to-head comparisons of the existing tools, using a standard set of archived or easily collected field samples, is needed**

Alternative Antigens/Platforms

Optimal diagnostic tools for LF programs may require the use of different antigens or platforms than those that already exist.

Two presentations to describe alternative antigens or platforms were given during this session. Topics and presenters were as follows:

1. “BLF Rapid” – Rahmah Noordin
2. “Wb123/Bm14 duplex” – Syamal Raychaudhuri

Key Points:

- BLF Rapid – lateral flow test using recombinant BmSXP antigen
- In laboratory evaluations, BLF Rapid was more sensitive than PanLF Rapid; diagnostic sensitivity ranged from 84%-100%
- BLF Rapid cross reactive with oncho, Mansonella and Brugia
- InBios Wb123/Bm14 duplex – lateral flow test using Wb123 and Bm14 antigens
- Relatively good concordance between ELISA and duplex prototype

Discussion points and OR opportunities:

- BLF Rapid not suitable for use in areas where multiple filarial infections are co-endemic
- Are Wb123 and Bm14 the most appropriate antigens to combine into a multiplex test?
- **Evaluation of BLF Rapid in programmatic settings is needed**

Sampling Methodology

The ubiquity of GPS-enabled smartphones, expanded coverage of remote sensing satellites, and increasing availability of open-access data sources have led to a dramatic increase in the types of data that can be collected and the amount of information that can be incorporated into decision-making. NTDs can learn from the sampling methods used in other sectors to improve our predictive maps, identify potential hotspots and optimize the use of field resources.

One presentation to introduce new possibilities for sampling strategies was give as follows:

1. “Spatial and Adaptive Learning” – Ben Arnold

Key Points:

- Advances in spatial information gathering and interpretation create exciting new opportunities
- We can better use the information we currently collect to inform future sampling and follow-up
- NTD researchers may be able to learn from other sectors that are using adaptive sampling to generate predictive maps with greater certainty across the entire geographic area as well as to identify potential transmission hotspots

Discussion points and OR opportunities:

- **Can adaptive sampling be used to identify hotspots of high antibody prevalence? And if so, are these hotspots associated with greater ongoing transmission?**

LF/Oncho Breakout Group

A small group was convened to discuss diagnostic needs for areas endemic for LF only or co-endemic for LF and onchocerciasis.

Discussion points and OR opportunities:

- BmR1 appears to decline quickly after MDA and may offer opportunities for surveillance
- Work being done to compare performance of Ov16 ELISA and Ov16 RDT; results of these comparisons are needed before making programmatic recommendations for LF/oncho overlap areas
- FTS is an adequate tool for mapping, monitoring and stopping MDA; need to determine how to follow up FTS positive children identified in TAS
- For LF surveillance, there may be a need for antibody tools instead of/in addition to FTS; current version of Wb123 RDT is inadequate and other RDTs (e.g. BLF Rapid) have not been field evaluated
- Performance characteristics of any RDT used for surveillance must be well-defined and may require target product profiles
- **Further investigation is needed in Brugia areas to determine the utility of monitoring BmR1 for surveillance; adult populations need to be tested over time**

- **There is a need to develop protocols for following up positive children identified in TAS; need to determine if a “2nd decision rule” strengthens interpretation of TAS results**
- **Sufficient quantities of samples are needed to conduct comprehensive evaluations of multiple diagnostic platforms; may require venipuncture collection**

LF/Oncho/Loa Breakout Group

A small group was convened to discuss diagnostic needs for areas endemic for LF, oncho and loa loa.

Discussion points and OR opportunities:

- FTS will remain the programmatic tool for starting, monitoring and stopping LF MDA; however the Loa cellscope will be used to rule out any FTS positives due to cross-reactivity from Loa loa microfilaremia
- Biplax will always be used where Loa is suspected to be endemic in order to assess the status of oncho and the Wb123 can be used to assess whether there is co-infection with LF and Loa
- In a research setting we need to collect all the information we can – including DBS for ELISA and/or multiplex testing
- **The discrepancy between RDT and ELISA needs to be explored in the context of loa-endemic areas; it is particularly important to better understand with wide range of Wb123 response observed across sentinel villages in Gabon**
- **What is the decrease in loa loa intensity over time following IVM + ALB vs. ALB alone?**