Ov-16 Meeting Notes
Neglected Tropical Diseases Support Center
Taskforce for Global Health
Decatur, GA, USA
May 2–3, 2016

Abbreviations

APOC	African Program for Onchocerciasis Control
CI	confidence interval
DBS	dried blood spots
DRC	Democratic Republic of the Congo
ELISA	enzyme-linked immunosorbent assay
HRP	horseradish peroxidase
IVM	Ivermectin
LIPS	Luciferase Immunoprecipitation Systems
MDA	mass drug administration
MF	microfilariae
NIH	U.S. National Institutes for Health
OCP	Onchocerciasis Control Programme in West Africa
OD	optical density
OEPA	Onchocerciasis Elimination Program for the Americas
OR	operational research
PCR	polymerase chain reaction
PES	post-elimination surveillance
PTS	post-treatment surveillance
QA	quality assurance
QC	quality control
RDT	rapid diagnostic test
ROC	receiver operating characteristics
SAC	school aged children
SNP	single nucleotide polymorphism
WHO	World Health Organization

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Introduction

The new World Health Organization (WHO) guidelines for stopping mass drug administration (MDA) and verification of elimination of human onchocerciasis require evaluating children under the age of 10 years using a serologic test for the detection of IgG4 antibody to the Ov-16 antigen. The designated sampling frame is designed to provide a 95% confidence interval (CI) that excludes 0.1%, and it assumes that the test is 100% sensitive and 100% specific. Recently, a rapid diagnostic test (RDT) for IgG4 antibody to Ov-16 became commercially available. Although the Ov-16 enzyme-linked immonusorbent assay (ELISA) has been used to make stopping decisions in the Americas and several foci in Africa, the RDT has not. It will be important understand how the RDT performs in comparison to the ELISA format in order to appropriately use the RDT for important programmatic decisions. The performance of the RDT has been compared to the ELISA in controlled setting using panels of samples with known infection status and in a programmatic setting in Togo. Both of these settings are more reflective of the hyper/mesoendemic setting. Little is known about how the RDT performs in settings with little to no transmission. According to the WHO guidelines, an operational research priority is to validate the use of the Ov-16 RDT for making the decision to stop mass drug administration for onchocerciasis (*see section 8.1.2.5*). An additional research question specified in the new guidelines is the need to understand the rate of sero-reversion of the Ov-16 antibody response. This information could be used by modelers to help determine potential new thresholds for stopping MDA. More importantly, understanding the dynamics of the antibody response may make it possible to use Ov-16 serology in community-wide or school-based evaluations (potentially added on to evaluations for other purposes) for post-treatment surveillance (PTS) or post-elimination surveillance (PES). Finally, despite the successes in the Americas, it is not really possible to measure a 0.1% threshold using the currently available diagnostic tests (both ELISA and RDT) as neither test is 100% specific. Although the new guidelines allow programs to use skin snip polymerase chain reaction (PCR) to evaluate potential false positive Ov-16 reactivity, it is counterintuitive to use a test that is less sensitive than the initial test used to exclude a diagnosis. In the absence of a confirmatory serologic test, the most likely way forward is to identify more realistic, evidence-based thresholds and sampling frames that allow for some potential false positives.

Because of these needs, the Ov-16 meeting was convened in order to examine several issues of programmatic importance.

Specific objectives were:

1) To examine the currently available data in which the Ov-16 RDT was compared to Ov-16 ELISA or skin snip in a variety of epidemiologic settings in Africa
2) To begin to examine the appropriate serologic threshold and age group for determining that it is safe to stop MDA for onchocerciasis
3) To examine what is currently known about the rate of Ov-16 sero-reversion

Key Findings and Discussion Points

1) RDT Performance: The Ov-16 IgG4 rapid diagnostic test (RDT) performed well in hyperendemic areas with sensitivity around 80% for detecting people with microfilariae (MF) present on skin snips in hyper and mesoendemic areas, which was the targeted sensitivity for the RDT. The Ov-16 IgG4 RDT had an unexpectedly low sensitivity of 40-60% for detecting people with MF on skin snips in untreated hypoendemic areas and in hyperendemic areas treated long enough to have significantly suppressed, if not eliminated, transmission. The sensitivity of Ov-16 IgG4 RDT compared to Ov-16 IgG4 enzyme-linked immunosorbent assay (ELISA) was >90% when compared in banks of specimens from people with known infection status (e.g. parasitologically confirmed from endemic areas) but was 40-65% when compared in areas that had transmission suppressed, if not eliminated. It is important to note that despite this unexpectedly low sensitivity the prevalence of RDT positivity was greater than skin snip microscopy except in an evaluation in Togo.

2) Validation of the RDT: The new World Health Organization (WHO) guidelines for stopping mass drug administration (MDA) and verification of elimination of human onchocerciasis specify that Ov-16 serology should be used to determine if MDA can be stopped. The guidelines also specify in section 8.1.2.5 that a priority for operational research should be the validation of the use of the RDT for making the decision to stop MDA. Given the results specified above in point 1, it is a priority to evaluate the RDT performance in settings of little to no transmission.

Key steps to allow the roll-out of the RDT for stopping decisions include:
- Selection of a harmonized ELISA protocol so that standardized, more easily interpreted comparisons of RDT and ELISA performance can be made
- Rapid evaluation of remaining RDTs from the studies that found decreased sensitivity to determine if there might be an issue with the manufacture of the test or the implementation of the test in the field
- Comparison of RDT performance in the field to performance in the laboratory using dried blood spots (DBS)

Depending on the findings of the aforementioned evaluation solutions may include:
- Adapting sampling protocols (e.g. increase the required sample size)
- Recalibration of the RDT (e.g. try to set a lower limit of detection
- Creation of RDT standardized operating procedures to minimize errors caused by use in the field
- Improvement of the manufacturing process

Additionally it will be important that a standardized protocol for quality assurance be implemented by all programs using the RDTs (see http://sites.path.org/dx/ntd/training-and-qaqc-materials/). It would be helpful that for the time being all groups using the RDT also collect DBS in case field results need to be compared either to the harmonized ELISA or to the RDT in a laboratory setting.

3) Development of biomarker detection (antigen or other) for the presence of adult female worms: Although several groups had initially been funded to work on development of a biomarker test for fertile adult female worms, this work has been de-prioritized. This is unfortunate, given the four major potential uses of such a test. These uses include:
- Use as a potentially more sensitive (and less invasive) test than skin snip PCR for the evaluation of actively infected individuals that could contribute to transmission
- Evaluation of response to treatment in trials of potential macrofilaricides
- Evaluation of parasite transmission in settings where the interruption of transmission has been accelerated by use of doxycycline, twice annual ivermectin, or vector control
- Evaluation of parasite transmission in hypoendemic settings where modeling suggests that transmission could be interrupted in less than 12–15 years

As programs attempt to accelerate the interruption of transmission in order to meet global elimination goals, the basic assumption that MDA continues for longer than the reproductive life span of adult female worms may be violated. In this setting, MDA may have to be continued despite interruption of transmission due to the limitations of Ov-16 serology. Despite the lack of sufficient funding, progress has already been made on the development of an antigen test. The identification of a funder for this endeavor would markedly accelerate the development of what could be a game-changing diagnostic test.

4) Mapping of hypoendemic areas: There was general agreement that serology is the tool that should be used for mapping hypoendemic areas. Skin snip microscopy would not be an appropriate tool for mapping hypoendemic areas given its low sensitivity.

Even skin snip PCR is less sensitive than serology, so serology is the way to go. The available data suggest that seroprevalence in children is not necessarily reflective of transmission in the community, so community sampling may be necessary. The appropriate threshold for the initiation of MDA is not clear. Although consensus was not obtained, a majority of participants agreed that 2% would be a conservative threshold given that models suggest that in hyperendemic areas this would be an appropriate threshold for stopping MDA. There was consensus that additional modeling, experience in the field, and data from vector collection could allow this threshold to be raised.

5) Re-evaluation of the serologic threshold for the interruption of transmission: Similar to point 4 above, modeling results suggest that 2% seroprevalence would be a conservative threshold for stopping MDA in many hyperendemic areas. The precise cut point would vary depending on the baseline intensity of transmission, so this cut point could be raised as more empiric data are obtained. The age seroprevalence curve using the RDT in Tanzania demonstrated flat seroprevalence of around 1% in children less than 16 years of age with an inflection point in adults older than 20 years old. The flat curve suggests that the 1% seroprevalence may be background noise and that transmission may have been interrupted. Data on the prevalence of infection in black flies are pending. As using an overly conservative serologic threshold could result in continuing MDA longer than necessary, it will be important to develop the evidence required validate a different threshold. Additional age seroprevalence studies in settings where transmission of onchocerciasis may have been interrupted in combination with vector data would help the development of the empiric data needed to support the modeling conclusions of a higher serologic threshold for stopping MDA.

6) Re-evaluation of the appropriate age group for evaluating the status of transmission: There is interest in expanding the age group used for making stop MDA decisions. A larger age cohort could make integration of evaluation with other programs more feasible, which will become particularly important if it is determined that serology, rather than vector collection, could be used for post-treatment and/or post-elimination surveillance. Including older children would make it easier for programs to obtain the sample sizes currently recommended for assessing the status of transmission. Including older children would be at the very least a more conservative estimate of transmission in that it would require for transmission to have been interrupted for a longer period of time for an area to pass the evaluation. Modeling suggests that the 5 – 14 year old age group may even be better at appropriately identifying the interruption of transmission than other combinations of age groups. The limited data available from recent studies (e.g. Tanzania and Togo) appear to be consistent with this finding. More empiric data are needed to identify the age group of children that best reflects the status of onchocerciasis transmission in an area.

Presentations and Discussions

Tom Nutman – History of Ov-16

Key points:

- The Ov-16 IgG4 serologic test was designed to be specific and early indicator of infection. Including a cocktail of antibodies to other antigens that were available at the time in addition to the anti-Ov-16 antibody did not improve the performance of the test.
- The test was evaluated in the Americas and in Africa; in adults and in children. It was determined that populations with high prevalence of MF had higher seroprevalence of anti-Ov-16 antibody. Children born after implementation of control measures had lower seroprevalence of antibody than those born before control measures were implemented. Lindblade et al. and Gonzalez et al. evaluated Ov-16 and found that it was a useful benchmark for transmission interruption.
- Data support its use for diagnosis, surveillance, detection of recrudescence, and the evaluation of the status of transmission.
- The Ov-16 IgG4 ELISA has been successfully used in the Americas to demonstrate the interruption of transmission despite a lack of quality assurance and quality control of the ELISA.
- An earlier version of the Ov-16 IgG4 RDT was demonstrated to have good sensitivity and excellent specificity in a variety of settings.

Key discussion points:

- Multiple comparisons between skin snip microscopy and Ov-16 RDT prototype have been made. The sensitivity of serology compared to mf in snips has been between 70 and 90 percent.
- The greatest difference between RDT and ELISA results in the early studies was that the ELISA was quantitative.

Paul Cantey – Guidelines for Stopping MDA and Verifying Human Onchocerciasis Elimination

Key points:

- The new 2016 guidelines require demonstration of <0.1% seroprevalence of Ov-16 IgG4 in children under the age of 10 years before stopping MDA.
- Blackfly evaluations, using poolscreen PCR are needed before stopping MDA, during PTS and during PES. The wording about blackfly evaluations for PES leaves room for other methods.
- The morbidity requirement has been dropped.
- Independent expert committees that review program data and provide recommendations to the ministry of health are a required part of the verification process.
- Priority research topics include the validation of the Ov-16 RDT for use in making the decision to stop MDA, the determination of the rate of Ov-16 sero-reversion, and the development of a standardized fly catching protocol.
- The evidence base for the use of Ov-16 serology is not very strong according to the WHO guidelines criteria for assessing evidence. Additional published data would help strengthen the evidence.
- The guidelines may be accessed through this link: http://www.who.int/onchocerciasis/resources/9789241510011/en/

Other points of interest about evaluation of children include:

- A random sample of the children is needed and it should be stratified using the local lower administrative unit (i.e. sub-district or smaller) according to the guidelines.
- Data need to be analyzable by age. The children evaluated need to be at risk for onchocercaisis. The lower age limit has not been set and that may vary from population to population.
- When there are fewer than 2000 eligible children, the guidelines specify how to do sampling – typically a census.
- The seroprevalence study needs to be done during the same quarter as blackfly evaluation and during the period when transmission intensity is highest.
- Currently skin snip PCR can be used to evaluate Ov-16 positive children if there are 10 or fewer positive children. However, the child should be off ivermectin for at least one year and the sensitivity of skin snip PCR is less than that of serology, so a highly sensitive confirmatory test would be helpful.

Other items of note:
- Entomologic and epidemiologic monitoring every 4-5 years is recommended.
- There is a need for regional labs that can perform the Ov-16 ELISA (at least until the RDT is validated) and that can perform O-150 PCR of blackflies.

Key discussion points:

- While the 2016 WHO guidelines state only that Ov-16 serology is needed, currently only the ELISA is considered a validated format. If that was not the intention of the committee, there may need to be some clarification made by the committee.
- Once there is enough evidence about the performance of the RDT to consider it to be a validated alternative it should not be necessary to change the guidelines given the language of the current guidelines.
- The switch from purposeful sampling to random sampling of the entire focus is new. There are data that suggest that the further you move from breeding sites, the lower the risk of transmission. Comparisons between purposeful sampling, random sampling, and perhaps a two stage process that includes both could be warranted. This is an area for OR.
- It was questioned whether more data on Ov-16 sero-reversion are needed. There is some disagreement on the rate of sero-reversion (see presentation by Vita Cama). In any case, this issue is specified in the guidelines, so until there is consensus that further development of our understanding of sero-reversion is not needed, it should be considered a priority.

Evaluation Ov-16 in Uganda, Ethiopia and the Democratic Republic of Congo (DRC) – Paul Cantey and Vita Cama

Key Points:

- The study characterized specimens from individuals in 3 hyperendemic foci with minimal exposure to ivermectin (IVM) MDA.
- The performance of Ov-16 serology in hyperendemic sites in East and Central Africa is similar to what has been demonstrated in previous studies.
- Sensitivity compared to snip PCR was 83-93%, with a decrease in sensitivity found in DRC in individuals infected with *Mansonella perstans*.
- Sensitivity and specificity of the Ov-16 ELISA determined by receiver operating characteristic (ROC) analysis was 88% and 99% respectively, compared with skin snip PCR and a panel of known parasitic infections in individuals from onchocerciasis non-endemic areas
- In all three sites serology identified more people as infected than both skin snip microscopy and skin snip PCR, even though not all MF-positive people develop OV-16 antibodies.
- Age prevalence curves, which should be interpreted with caution due to small numbers in the youngest age group, demonstrated a rapid rise in seroprevalence in Uganda, with seroprevalence in the 11 to 20-year-old age group approximating prevalence in the > 20-year-old population. The rise in seroprevalence was

slower in the other two sites, where the adult seroprevalence was not reached until the 21 to 30-year-old age group.

Key discussion points:

- There are several versions of the ELISA being used. In order to create standard comparisons between ELISA and the RDT, a harmonized protocol will be needed. The CDC ELISA is similar to the OEPA ELISA, but some modifications have been made.
- It does not seem practical to promote the use of ELISA in Africa. In addition to development of a harmonized protocol, there have been issues with getting needed materials into countries. There will also be issues of QA and QC that would be easier to deal with using the RDT. PATH is currently working on an ELISA kit for Ov-16, and it would be very helpful to have input on what is needed performance-wise.

Allison Golden – PATH Ov-16 Experience

Key points:

- PATH has evaluated the performance of its ELISAs and its RDT and found them to be sensitive (72-88%) and highly specific (100%) in laboratory settings.
- The sensitivity of its ELISA was unexpectedly low (compared to skin snips) in a field setting (60–70%) in Togo.
- Age prevalence curves were created as part of the evaluation in Togo and appeared to differ when categorized into 3 groups of village seroprevalence (<15%, 15-20%, and >20%).
- Performance of the tests in the field is expected to deviate from performance in the laboratory. PATH will continue to collaboratively collect data in order to understand this difference in performance.
- PATH supports the idea that data should continue to be collected on Ov-16 ELISA and RDT to validate their use for decision making.
- PATH will be collaborating with the program in Senegal to evaluate the performance of the commercial RDT in a setting where transmission has likely been interrupted.
- PATH is preparing a QA program which includes guidelines, training, control panels. The program will help generate confidence in the data derived from the RDT, which is needed particularly as the RDTs get subjected to a lot different temperatures, conditions and users. The program will need to be supported in the long term by an organization other than PATH.

Key discussion points:

- It is unclear why the sensitivity of the horseradish peroxidase (HRP) ELISA was low in Togo. It could be an issue of sample size since there were fewer than 50 individuals with MF. It could be something else. The analysis is limited by the lack of a good reference assay for skin snip negative individuals.
- It was pointed out that a commercially-produced RDT is available. There are data to support its use. There is a QA/QC program available for the RDT but not ELISA. The RDT could cost less per test than what a potentially standardized ELISA would cost; the RDT costs $1.20 per test. The RDT requires little to no expertise, though training personnel to use the test correctly is important as other programs have had difficulty with generating repeatable results with other rapid card tests.
- The RDT results may be more repeatable by using DBS, and this is an area of active study. Using DBS for the RDT is an off-label use that has not been sufficiently validated. More study should help determine if the Ov-16 RDT can be used with DBS.
- The general agreement was that the goal is to have an RDT, but we may need more data prior to abandoning a format (ELISA) that has been demonstrated in the Americas to be useful for making treatment stopping decisions.

Joseph Oye – The experience in Cameroon

Key points:

- The site of evaluation was the Massangam health district, which has had persistent transmission despite >15 years of MDA in the Makoupsap transmission zone.
- RDTs in children and snip in adults and some children were performed to assess the impact of the program and identify problem areas.
- MF prevalence in people over the age of 5 years was 12.9%.
- Ov-16 prevalence by RDT in children 3-15 years old was 9.7% and was highest in communities with the highest prevalence of MF in skin snips performed in older children and adults.
- In the small subset of children 6-15 years old who had both skin snip and RDT results (n=33), RDT identified only 95% of those with MF on snip.
- In the larger sample the RDT identified more infections in children 6-15 years old than skin snip.

Key discussion points:

- RDTs were not performed in all people with skin snips so the comparison between snip and RDT could only be done in a small subset of the population.

- The reason for the persistent transmission is unclear. There might be a group of systematic non-compliers, but there are also other project areas nearby.

Olabanji Surakat – Experience in Nigeria

Key points:
- The study was performed in mesoendemic area with >10 years MDA.
- The prevalence of positive results was 17.3% by skin snip microscopy, 9.3% by Ov-16 RDT (2.9% in children 5-9 years old), and 18.8% by Ov-16 ELISA.
- Only a subset of people had skin snips (n=192) and ELISA (n=589) performed so the numbers should be interpreted with caution.
- In the subgroup that had both ELISA and RDT, the prevalence was similar though there were more people with positive ELISA results and the RDT result was positive only in 72% of those with positive ELISA results.
- In the smaller subgroup that had both RDT and snip, RDT identified 80% of the participants with MF in skin snips.
- Age prevalence curves demonstrated a rise in seroprevalence by RDT until the 25 to 34-year-old age group (which had a 14.8% seroprevalence), after which seroprevalence declined somewhat.
- A similar trend was seen in prevalence of microfilardermia in the subset of people who had skin snips performed, with the peak prevalence of 28.4% in the 25 to 34-year-old age group.

Key discussion points:

- A vector collection has taken place in the areas and around 6,000 flies have been collected; collection is on-going.
- There was a discussion about why there was a dip in seroprevalence in some of the older age groups (35-54 years old). One contributing factor could be women of child bearing age as they cannot take ivermectin when pregnant, though this is an unlikely explanation. It was suggested that age prevalence curves by gender be developed. It was also suggested that this could be related to occupation, though they would more likely explain higher prevalence in men, not an age specific relationship.

Maria Rebollo – Mapping with Ov-16 from the AFRO Mapping Project

Key points:

- Several large evaluations using skin snips and Ov-16 RDTs enrolled more than 20,000 people in untreated areas thought to be hypoendemic for onchocerciasis

and in countries where elimination may have occurred. These studies were funded by the Bill and Melinda Gates Foundation and USAID.

- In Gabon in areas that were found to be hyper/mesoendemic, the RDT had 78% sensitivity for MF on skin snip microscopy; as the RDT was positive in individuals without MF on microscopy as well, overall the RDT identified more people as infected than skin snip.
- In Gabon in areas that were found to be hypoendemic, the RDT was only 46% sensitive for MF on skin snip; the RDT identified many more infections than the skin snip.
- In a hyper- and mesoendemic setting, the prevalence of both MF and Ov-16 in school aged children (SAC) was similar to adults. For Ov-16, 63% of children aged 5-14 years old were positive by RDT and 70% of people >15 years old were positive. In the hypo-endemic areas the prevalence of Ov-16 seropositivity in SAC was not similar to adults; the prevalence of Ov-16 RDT positive was 3% and 11% respectively.
- Comparisons in DRC and Nigeria (untreated hypoendemic) and Mali, Guinea Bissau and Malawi could not be made due to few to no positive skin snips.

Key discussion points:

- There is no information on co-infection with other filarial infections, but some skin snips were preserved so PCR could be performed. In many areas no one was found positive by RDT.
- It was suggested that one could recalculate the skin snip results, adjusting for the expected sensitivity of the snip compared to the Ov-16 results. The snip results just are not very reliable in the hypoendemic area. Ov-16 appears to be the best way to map the infection with current tools.
- The 50–60% sensitivity of the RDT for detecting individuals with MF on skin snip microscopy is a bit concerning. There was some discussion about whether zoonotic onchocerciasis could cause a positive Ov-16 (unlikely, particularly as IgG4 was selected to avoid cross-reactivity) and that Ov-16 can precede snip positivity as an explanation for some of the discrepant results. However, that would not explain the large number of snip positive individuals with negative RDT results. Although a portion of the population may never develop a response, that proportion is likely to be in the 5-10% range.
- Cross-reactivity discussions continued. It was pointed out that although all of the filarial parasites have Ov-16 homologs, they are sufficiently different as to make cross-reactivity a non-issue, particularly if one uses IgG4. The National Institutes of Health (NIH) has created a variome with *Onchocerca* from locations around the world. A few single nucleotide polymorphisms (SNPs) have been found in Ov-16 but they were not in areas that are antigenic. Therefore it appears that the

test should be useful for strains throughout the endemic areas (although exceptions could occur and would need to be investigated if they arise).

- A question was asked about whether Ov-16 was stage specific. The response was that while the L3 and L4 are the major transcriptionally active stages for Ov16, there is certainly transcription and protein production at all stages of the parasite. There was a fair amount of adult-derived protein that is also driving the production. This is not a stage-specific antigen

Experience in Côte d'Ivoire – Chris King (via WebEx)

Key points:

- These data come from a clinical trial of triple drug therapy (IVM+diethylcarbamazine+albendazole) for lymphatic filariasis (LF).
- There was no evidence of cross-reactivity with LF.
- Although the numbers were quite small, it was reassuring that all skin snip positive individuals had a positive RDT result.
- One MF positive person had a negative RDT at 20 minutes but a positive RDT at 24 hours; two others also converted to positive at 24 hours.

Key discussion points:

- It was asked whether the Ov-16 RDTs that were read at 24 hours should be considered false positives or false negatives since the test should be read at 20 minutes. As one of the individuals had MF on skin snip, perhaps the reading time should be longer than 20 minutes. Different time points have been evaluated in the past, and a low level of conversion has been demonstrated for longer reading times. Sometimes in the lab a very faint positive that is difficult to read at 20 minutes might be easier to read after 24 hours. Typically, however agreement between the 20-minute and 24-hour reading is greater than 90%. If the problem continues to be identified, especially in the field, a 30-minute recommendation could be considered. As of the meeting, 3,500-4,000 RDTs had been performed in Cameroon as part of the Test-and-Treat study with readings at 30 minutes and 24 hours. So far the prevalence values using the different time points have been virtually identical, but at the individual level there was discordance in relatively few participants in both directions. As DBS were used in the Test and Treat study these results might not be comparable to the results when whole blood is used in a field setting. Other investigators have evaluated RDT results at various time-points and found that there were few changes.

Rachel Bronzan – The Experience in Togo

Key points:

- There has been a longstanding program in Togo that started with vector control and then added IVM MDA. Two studies from Togo were presented:
- Study 1:
    - >7,000 people ≥ 5 years old in 49 villages that had received > 15 years of MDA were evaluated with both skin snip and RDT
    - 1.5% of skin snips were positive by microscopy and 7.8% of RDTs were positive.
    - The RDT was 53% sensitive for MF on skin snip; all 7 children under 15 years old with a positive skin snip had a negative RDT
    - There were few positives by either method in children under 15 years of age (<5%), though RDT consistently identified more people.
    - Seroprevalance by RDT in older adults was around 15%, which was reassuring.
    - Togo needs to complete skin snip PCR and Ov-16 ELISA on DBS in order to complete its evaluation of the diagnostics.
- Study 2:
    - Children ages 6–9 years in schools across Togo were evaluated by RDT; 2578 children in 9 districts had DBS in addition to RDT and 460 of the DBS were analyzed by ELISA.
    - 60 (0.7%) of 8961 children across Togo were positive by RDT
    - 72 (15.8%) of 458 RDT-negative samples were positive by ELISA
    - Troubleshooting is ongoing to determine the cause of the discordance between RDT and ELISA; reassessment of the ELISA cut point using the EM methodology described by Katie Gass reduced the percentage of RDT-specimens that were positive by ELSIA to 13.9%.
    - 37 RDT negative/ELISA positive samples had the RDT rerun using DBS in the lab; the results of these RDTs were all negative.

Key discussion points:

- There was a concern about the lack of concordance between RDT and ELISA results. One issue of concern was that GST reactivity may cross-react with *Schistosoma mansoni*. As far as the Togo data were concerned, however, there was no correlation between schistosomiasis and Ov-16 ELISA positivity.
- There was much discussion about different steps of trouble shooting the ELISA. In the end, the discussion focused on developing a common methodology for ELISA.

Paul Cantey – Experience in Tanzania

Key points:

- An age-stratified study was performed in order to compare test performance in a primarily meso/hyperendemic area that had received 15 years of MDA with ivermectin plus several years of vector control and several years of ivermectin outside traditional campaigns
- Results for skin snip, RDT, and ELISA were presented; no one under the age of 6 years had a skin snip taken and the ELISA results are not ready for this age group.
- 1.3% of people had nodules on examination
- 0% had MF identified by skin snip microscopy, and 5.5% had a positive RDT result.
- Age group prevalence of positive RDT results was:
    - ≤ 5 years old   0.5% (95% CI: 0 – 1.8%)
    - 6 – 10 years old   0.4% (95% CI: 0 – 1.4%)
    - 11 – 15 years old   0.8% (95% CI: 0 – 2.2%)
    - 16 – 20 years old   2.2% (95% CI: 0 – 5.7%)
    - > 20 years old   10.5% (95% CI: 4.8 – 16.2%)
- ELISA found a population prevalence of 14.6% compared to 6.5% by RDT (children ≤ 5 years old not included)
    - Despite this difference the age group prevalence in children was similar by both methods.
    - The sensitivity of the RDT for positive ELISA was 42%.
    - The sensitivity of the ELISA for positive RDT was 86%.
    - 10 out of 12 people with nodules had positive ELISA result whereas 0 out of 12 had positive RDT result.
- The Tanzania site would fail current WHO guidelines for stopping MDA, although the age prevalence curves were flat in the 3 youngest age groups.
- Although complete ELISA data, skin snip PCR, and vector pool-screen PCR data are needed, these results make one question whether the serologic threshold is appropriate, particularly as the positives in children seen in this study could just be background noise
- The age seroprevalence of Ov-16 in adults >20 years old was lower than expected if the antibody response was in fact lifelong.

Key discussion points:

- There was a discussion about the importance of vector sampling. If there is transmission ongoing, you should see something in the flies. If all of the flies are negative, it doesn't necessarily indicate that transmission has been interrupted, but if they're positive it is clear that transmission is ongoing. What the flies don't show you is the status of adult female worms (and neither does Ov-16). If there

remain viable adult females, recrudescence of transmission could occur if ivermectin is stopped. Currently, we have to make sure the program has lasted longer than the average lifespan of the worms, stop IVM when appropriate, and then perform PTS. The current vector collection protocols assume that flies bite at random. This has been shown not to be true for mosquitoes for some diseases. Models suggest that there is heterogeneity in biting of black flies as well.

- More discussion about the RDT occurred. It was stressed that there could be lot to lot variability in the RDT or issues with how the RDT was handled that could influence results. For that reason QA/QC is very important. In regards to the Tanzania study, the RDTs were stored in a climate controlled building for several weeks and then at ambient temperatures a few more weeks, though this was at an elevation where temperatures were mild. When the ELISA/RDT discordant results were examined in a preliminary analysis, quite a few of the discordant results occurred more than 2 standard deviations away from the ELISA cut point, so although the sensitivity of the RDT for positive ELISA improved, it remained low.

Discussion – Ov-16 for mapping

A discussion about using Ov-16 for mapping areas of low transmission yielded the following important points.

- Experience from work with testing for lymphatic filariasis found, as expected, that test performance changes when the test is moved from hyperendemic to low prevalence settings. There is more discordance between different tests and sensitivity typically decreases. This is not unlike what we are seeing with Ov-16.
- As different protocols for the Ov-16 ELISA have been used to evaluate the performance of the Ov-16 RDT, it will be important to harmonize the protocols so that the comparison is the same. Any tweaking of the RDT sensitivity will come at a cost in specificity, so we need to be careful. It will not be realistic to deploy the ELISA throughout Africa, so we need to focus on how best to use the Ov-16 RDT.
- As part of the effort to deploy the Ov-16 RDT it will be important to minimize errors caused by inappropriate use of the RDT or by poor quality RDTs. It is essential that a good QA/QC system be implemented and that standardized training materials be available and used consistently. All of the other techniques (e.g. ELISA) use different protocols, primers, machines, reagents, etc. As long as there is a good QC system in place and the ability to evaluate batches of tests with standard curves, the results from RDTs should be comparable across sites.

- A discussion took place about whether or not intensity of antibody response (e.g. titers or intensity of the optical (OD) reading) was related to microfilarial density. The general consensus was that there is no correlation.
- Even if the RDT sensitivity cannot be changed at this point in time, it will be important to understand how the RDT performs compared to other tests so that sampling strategies can be adjusted to compensate for decreased sensitivity in low prevalence areas. Mapping is on-going and we do not want to miss areas that require treatment.
- It might be helpful to collect flies in some of the areas where the RDT is deployed for mapping to see if the flies indicate that transmission is on-going and at what level of RDT positivity. Fly collection in this setting will be more challenging, as generally the location of breeding sites is less apparent.
- The lack of a gold standard diagnostic test makes the evaluation of the RDT and comparison to the ELISA even more challenging. Both of the serologic tests are limited by the presence of 5-10% of the population that does not mount an antibody response to any given antigen. Serologic tests cannot be used to distinguish patent infection from previous infection (except in younger age groups). Although skin snip PCR is more sensitive than skin snip microscopy, a negative result on skin snip PCR does not completely exclude patent infection (it is less sensitive that serology). Skin snip PCR is less sensitive than serologic testing so it should not be considered a confirmatory test.
- There is now a potential cocktail of antigens that may allow us to test individuals in a way that is both highly sensitive and highly specific. It may possible to compare RDT performance to the cocktail so that the RDT performance can be clearly defined and then protocols adapted to compensate for any issues in performance. At the very least this should eliminate the issue of low sensitivity of comparison tests and the issue of a percentage of the population not responding to a particular antigen.
- Although it appears that younger age groups could be used for mapping in mesoendemic and hyperendemic areas, the increase in Ov-16 reactivity in children was much slower in hypoendemic areas in Gabon, so it appears that community-based surveys that include all age groups are likely going to be required in these areas.
- There was a discussion about the appropriate threshold for starting treatment in hypoendemic areas after mapping. Should the 0.1% threshold for stopping mass drug administration in meso and hyperendemic areas be used or should the serologic threshold for starting treatment in low transmission areas be higher such as 1 or 2%? As we cannot really measure 0.1% with the available tools, should we focus on a measurable threshold? A majority of people thought that 2%, given that modeling suggests that this would be the breakpoint of

transmission in many hyperendemic areas, would be a conservative point to start with.

Experience in Senegal – Achille Kabore

Key points:

- Two study sites: Kedougou (an area that has received 24 rounds of MDA) and Kolda/Tambacounda (an area that has received 17 rounds of MDA)
- They performed skin snip microscopy and Ov-16 ELISA from blood collected on DBS (CDC ELISA in Kedougou and Smith College ELISA in Kolda/Tambacounda)
- In Kedougou (~1000 people) there was 0.1% prevalence of MF on skin snips and 6.9% by ELISA.
- In Kolda/Tambacounda (~2800 people) there was 0.9% prevalence of MF on skin snips and 1.8% by ELISA (with another 1.5% indeterminate)
  - o 5 of the 9 with positive skin snip were younger than 15 years old.
  - o There was little evidence that the infected individuals were migrants.
- Two entomologic studies with at least 6,000 flies analyzed have found no infected flies

Experience in DRC/Gabon – Nils Pilotte

Key points:

- The study took place in 3 hyper/mesoendemic areas in DRC that had received around 10 years of IVM MDA.
- There were 820 people with results on ELISA and a prototype of the Ov-16/Wb123 Biplex RDT.
- The HRP ELISA was used; standard curves and a 4-parameter logistic regression model were used to create an inferred concentration for the OD readings.
- The prototype Biplex RDT identified 61% of the individuals with positive Ov-16 ELISA results. Of note, this prototype was tuned for higher Ov-16 specificity.
- The ELISA identified 83% of the positives on the Biplex RDT
- Age curves were similar for both tests in all 3 sites, with the fewest positives on both tests in Kiri, more positives in Kasongo, and an age-related increases in positivity in Punia

- o Interestingly there were children less than 5 years old who had positive ELISA results in both Punia and Kiri, but no child under 5 was positive by Biplex RDT in those two sites.
- There were significant issues with the physical condition of the DBS, which is concerning given:
  - o The desirability of DBS both for use with RDT and ELISA.
  - o The risk that small changes in numbers of positive test results could change the determination about transmission in an area.
- 111 samples from Gabon in which discordance between RDT and skin snip were evaluated by ELISA; only 22 had measurable antibody by ELISA creating concern for false positive skin snip microscopy.

Key discussion points:
- The discordant samples from Gabon were selected out of a much larger pool of specimens from Gabon (they represent ~2% of the sample). Because a certain percentage of people (5-10%) will not be able to mount an antibody response to a particular antigen, it is possible that this sample could just represent that group. A second test that uses a different antigen would be helpful to sort these out.
- The DBS that were felt to be suitable for ELISA based on the visible condition of the DBS, despite the poor condition of some of the other DBS collected, were not evaluated to determine if there was less than expected antibody on the DBS in good visible condition. The investigators have left over DBS for many of the individuals so that evaluation can be done to demonstrate the presence of antibodies in general on the DBS that were in good visible condition.
- A discussion ensued about why we are not simply using RDTs at this point in time. The ELISAs are not standardized and will vary between protocol and lab. The RDT is standardized for all users and comes with QA/QC options. Although the RDT is standardized, it was developed using tests that are not. Additionally, the new WHO guidelines specifically state that the RDT needs to be validated for use. Despite issues with Ov-16 ELISA and the characteristics of the tests in different labs, it is the only assay that has been used to verify the elimination of onchocerciasis. The RDT will have to be compared to this test.
- Once the difference in performance of the ELISA and RDT is understood then protocols or sampling frames could be adjusted, assuming samples sizes don't get too large, to compensate for differences in test sensitivity. Experiences with RDTs for LF suggest that RDTs are not as simple and straightforward to use as thought. Adding a DBS to the RDT so that the DBS could be archived and compared later, if needed, to a standardized ELISA could be a very useful exercise. Additionally, as many groups at this meeting already have archived

samples from a variety of settings it should be possible to quickly put together an evaluation using a standardized ELISA and the RDT.

- Finally, it was pointed out that despite the differences in the Ov-16 ELISAs, they have all been shown to be more sensitive than the RDT in some of the low transmission settings presented at this meeting.

Paul Cantey – Data Summary

Key points:

- ELISA has a high sensitivity in hyper and mesoendemic settings (80-90%) for individuals with MF on skin snip.
- RDT has a slightly lower sensitivity (closer to 80%) for MF on skin snip but still performs fairly well in the hyper and mesoendemic setting.
- The primary issue with the RDT appears in the hypoendemic setting, which is the setting in which we need most to use the RDT.
  - Other than in Cameroon, the RDT had <60% sensitivity compared to skin snips in this setting.
  - RDT performance in Cameroon is hard to interpret, it is likely that some areas still had high transmission and thus sensitivity was higher than expected (94%); small numbers in the sample may also have influenced the result
- Comparison of different ELISAs and the RDT shows a similar lower concordance of RDT results with ELISA results in low transmission settings (40-65%).

A way forward for the RDT

- There was general consensus that Ov-16 serology is the best tool for making the decision to stop MDA. And that Ov-16 ELISA, despite its limitations, has been successfully used to verify elimination in the Americas.
- It appears that the RDT correlates well with ELISA in meso- and hyperendemic areas. This correlation does not hold in the low transmission setting. Although there is an issue with different ELISA protocols being used, all of them have shown that the RDT fails to identify many ELISA positives and performs less well than ELISA when compared in sera from individuals with MF-positive skin snips.
- Even though the RDT did not perform as well as one would have hoped, as it was not as concordant with positive skin snip results in all individuals, it did identify more infected individuals than the skin snip. It might be possible to adjust sampling to compensate for an RDT that is less sensitive than the ELISA (assuming that the decreased sensitivity is demonstrated to be inherent to the

RDT and not an issue related to use in the field, problems with a particular lot, etc.).

- Rather than harmonizing ELISAs, which could be difficult, perhaps it would be better to either agree upon one ELISA protocol for RDT evaluations or compare all 3 ELISAs with the RDT. It would be challenging to develop capacity in multiple laboratories for the Ov-16 ELISA and would be challenging to analyze all the blood specimens needed for programs. The RDT really is the tool that needs to be widely available. As we already have a large repository of DBS taken in a variety of epidemiologic settings that could be used to compare the RDT, it should be fairly straightforward to do a well-controlled comparison in several laboratories using the same set of specimens in order to determine if the low sensitivity of the RDT is found by all protocols and then adjust sampling strategies to compensate for the lower sensitivity.
- Following age groups longitudinally over time could also be helpful as we will be able to see how many people with positive test results 'migrate' into the indicator age group over time. This would give programs insight into the impact that MDA has had on transmission during the last few years.

Katie Gass – Measuring 0.1%

Key points:

- The 0.1% threshold comes from the OEPA experience but it is not based on any particular data.
- Measuring the threshold put forth in the onchcocerciasis elimination guidelines and programmatically implemented in OEPA could be improved upon:
  - The sample size calculations appear to have assumed an infinite population; for most foci the sample required may end up being smaller.
  - The sample size calculations do not address power; they have very low power (~20%) making it difficult to detect programs that are performing well.
  - The sample size calculations do not adjust for sensitivity or specificity of the diagnostic test.
- Increasing power to 75% to detect a program that is performing well would require census sampling in populations <8,000.
- Using a test that is 80% sensitive would add 25% to the required sample size.
- In the absence of 100% specificity almost no focus would ever pass.
- If the threshold were 1%, the maximum sample size would be in the 1500 – 3000 range with up to 18 positives allowed, assuming a test that is 80% sensitive and an evaluation with 75% power.

- If the threshold were 2%, the max sample size would be in the 800 – 1700 range with up to 20 positives allowed, assuming a test that is 80% sensitive and an evaluation with 75% power.

Key discussion points:

- The threshold won't be the same for every focus as the threshold for interruption, according to the models, is dependent on baseline endemicity. As many programs don't have that information, seroprevalence of Ov-16 could be used as a proxy estimate of the baseline status. A more complete understanding of the dynamics of seroreversion would help programs use this proxy measure.
- It appears that blackflies become more efficient at transmitting the parasite when prevalence of the infection decreases due to density dependent uptake of MF by the blackflies. So as we attempt to adjust the threshold we will need to know the prevalence of infection in blackflies.
- As the models suggest different elimination thresholds depending on baseline endemicity, it was suggested that the modelers try to investigate several categories of baseline endemicity and determine the threshold for each of the categories, as a unique threshold for each focus would be too complicated. It was suggested that we could possibly use archived samples from the Americas to examine the threshold issue rapidly.

Cut point determination

Key points:

- The expectation maximization (EM) method to create mixture models to determine cut-points for diagnostic tests was suggested as a robust methodology to standardize the creation of diagnostic cut points for ELISA assays (e.g., Ov-16).
- Basically the method identifies distributions of results in the populations being tested. The EM method allows for the determination of cut-points that separate out these distributions based on a level of certainty that can be adjusted by the user. An indeterminate zone is usually recommended to more accurately reflect the degree of certainty with which points are classified; values falling close to the cut point will be classified as 'indeterminate' because they have a relatively similar change of belonging to the positive or negative distributions.
- When more than two distributions best fit the data, it can be difficult to determine which distributions should be considered positive and negative; the researcher is often required to make a best judgment call in such circumstances.

Key discussion points:

- The group discussed using the EM mixed model method to re-evaluate the cut points for the available ELISAs. This will likely need to be done for different epidemiologic settings (e.g. hyperendemic and hypoendemic).
- Although this was not discussed in the meeting, it was noted that if the harmonized ELISA uses an indeterminate zone, this should be taken into account when comparing the ELISA results with RDT results, as the RDT give only positive or negative results.

Vita Cama – Duration of antibody responses

Key points:

- The studies were performed in three settings: PTS period, after disappearance of the vector, and early in a program that has been distributing IVM twice per year.
- All 23 children who were positive by Ov-16 ELISA in an area where the vector was eliminated had sero-reverted by 6 years.
- In area which had completed PTS, the median OD reading had decreased to a value that was below the positive threshold. Models of the decline in OD suggested a 2.25-year half-life of the antibody response, with the caveat that some of the data comes from time points in which transmission could still have been occurring.
- In an area with active transmission, the OD reading of >300 individuals with known OD readings in 2012 had decreased by 50%. Models of the decline suggests a 1.5-year half-life of the antibody response.

Key discussion points:

- It was pointed out that the half-life seems short given the length of the antibody response in other individuals. As the analysis is preliminary, the time frame may be adjusted. There was also discussion about why some individuals maintained a high response for >15-20 years. Although this question could not be answered it was pointed out that compliance with IVM and disappearance of MF in the skin as well as the persistence of adult antigen in nodules even after adult worms die could impact the length of the antibody response. Although some individuals may remain positive for decades or more, it appears that in communities many individuals could sero-revert.

Wilma Stolk – Modeling

Key points:

- The ONCHOSIM model rests on a variety of assumptions:
    - A person becomes Ov-16 positive as soon as the criteria for conversion are met
    - The diagnostic test is 80% sensitive and 99% specific
    - No sero-reversion
- Depending on assumptions, it appears that early on MF prevalence is higher than Ov-16 prevalence though this changes as MF prevalence declines.
- The time to elimination depends on baseline endemicity and IVM coverage.
- There is a relatively stable relationship between the modeled value of MF prevalence or Ov-16 seropositivity measured1 year post-MDA and the modeled probability of elimination.
- In areas with high baseline endemicity, ONCHOSIM suggests that a post-MDA seroprevalence of 2-3% would be associated with a high probability of elimination.
- In areas of low baseline endemicity, the model suggests post-MDA seroprevalence of 5% or perhaps higher would be associated with a high probability of elimination.
- ROC analysis of many different simulations suggests that the 5 to 14-year-old age group, when compared to other groups of children under 15 years of age would give the most information about the status of transmission and that the 0-4 year olds would provide the least information.

Key discussion points:

- Much of the data for the threshold below which transmission does not recrudesce after stopping interventions come from the Onchocerciasis Control Program in West Africa (OCP) data. As much of the program was based on vector control, MF cut points determined from these data may not be equivalent to cut points in the setting of IVM use, where the drop in MF prevalence is an effect of the IVM and not a result of the death of adult worms. However, if suppression has gone on long enough the situations might be similar as the adults would have died as well. There were some areas in OCP that did not use vector control that were able to stop treatment. It would be important to review the specifics of those examples.
- If the RDT is the test of choice and the sensitivity of the test in the transmission assessment context is shown to be less than 80%, then the models will have to be re-run to identify the appropriate threshold.

Maria Gloria Basáñez – Modeling

Key points:

- Catalytic models can be linked to the EPIONCHO model and used to estimate the force of infection, which can be used to assess impact of interventions in the past by examining cross-sectional data as long as all age groups in the population are included in the sample.
- Sampling all age groups will allow for the assessment of sero-reversion rates.
- Models based on seroprevalence data can be used to predict thresholds in a particular age group that might be indicative of the interruption of transmission. As these thresholds will vary based on the dynamics of the setting, this would need to be done in a variety of settings.
- Currently available statistical and dynamic models could be used to assess sensitivity and specificity of diagnostic tests in the absence of a gold standard using Bayesian approaches, to estimate temporal changes in seroprevalence, adjust the African Programme for Onchocerciasis Control's (APOC) skin snip data from recent mapping activities, and assess thresholds for the interruption of transmission and the appropriate age group in which to measure the threshold.

Key discussion points:

- Some people felt the models are too cumbersome for programmatic use. It would be challenging to create thresholds for all foci and to perform large numbers of population-based serosurveys. It might be feasible to use sentinel sites, where surveys would be implemented on a routine basis to feed into some of the models and generate information for broad categories of programs. For other diseases (e.g. malaria), the models have worked well early in the programs but less well in the low transmission setting. It is unclear why the models perform less well in hypoendemic settings. It could be that factors such as non-random blackfly biting patterns, spatial clustering of transmission, population size or coupling between populations need to be taken into account. This needs to be investigated, and it is projected that the onchocerciasis teams in the NTD Modeling Consortium will work this issue on in the coming months.

Discussion about threshold of interruption of transmission and age group to evaluate

- Determining the appropriate serologic threshold(s) of transmission so that the decision to stop mass drug administration is based on robust information is a key OR question. The current threshold of 0.1% is both difficult to measure and

difficult to achieve. Using this threshold may result in wasting resources as programs are forced to continue treatments that are unneeded.

- Modeling results from ONCHOSIM and EPIONCHO suggest that a higher threshold, such as 2% according to ONCHOSIM, may be appropriate in many hyperendemic settings. This threshold could be tested in appropriately designed studies with adjustments made for the expected sensitivity of the RDT.
- In addition to reexamining the serologic threshold, the sampling strategy and the appropriate age group for sampling need attention. The current cluster-sampling methodology that is used by lymphatic filariasis may not be appropriate for onchocerciasis, given that the risk of infection in hyperendemic areas decreases as distance from the blackfly breeding sites decrease. However, this strategy might be sufficient and operational research could compare cluster sampling to community-based random sampling to determine if both methods result in the same conclusion. As modeling suggests that the 5-14 year old age group would be more informative than any of the other age groups, changing the age group evaluated should be examined and compared. As increasing the age of children produces a more conservative estimate of transmission (a program would have had to have suppressed transmission for a longer period of time in order to pass an evaluation), it should be acceptable to use older children. However, the upper age limit of the group included will be a function of the age of the program (e.g. 15 year olds should not be included in an evaluation of a program that is only 12 years old.)
- A sensitivity analysis of the serologic thresholds for transmission should be performed to increase our confidence in the 2% threshold that would be used for hypothesis testing.
- The RDT should be used in conjunction with DBS until we are certain that we no longer need DBS for additional testing (e.g. ELISA).
- The RDT should be evaluated in the mapping setting, the assessment of transmission setting, the monitoring and evaluation setting, and the post-treatment surveillance setting so we understand its performance for any potential use.
- Vector collections are needed.

Tom Nutman – Running RDTs using DBS/Experience from TnT

Key points:

- TnT trial has tested >15,000 people in Okola district in Cameroon to see if safe to treat with IVM, and 95% were able to be treated.

- Those with a MF count for *Loa loa* >8,000 MF/mL will be tested with RDT and confirmed with skin snip prior to being offered doxycycline.
- Blood for the RDT was collected on DBS and then eluted for use in PATH's RDT buffer, and run in the RDT, which was read at 30 minutes, 24 hours, 1 week, and 2 weeks.
- ~ 4,000 RDT have been performed so far. 25% of people were positive at 20 minutes and slightly fewer than that at 24 hours. There was not relationship between RDT positivity and presence of *L. loa* or *M. perstans* MF.

Key discussion points:

- The site was either mesoendemic or hypoendemic by rapid epidemiologic mapping for onchocerciasis and 25% of people are positive by RDT.
- The data will be examined to see if there was a correlation between a positive RDT and a Mazzotti reaction.

The *Onchocerca volvulus* Genome – Tom Nutman

Key points:

- The sensitivity of Ov-16 is an issue, so NIH began exploring other antigens that could be used to increase sensitivity. RNA sequence analysis was performed to identify genes specific to particular stages of parasite development. Proteomic analysis was performed as well. Diagrams were created to determine genes and proteins specific to one stage or common to multiple stages. This allowed the determination of both the presence of the transcript and the abundance of the protein by stage of development.
- More than 1,200 genes were examined in a process to identify vaccine candidates. Antibodies to about 8 antigens were found that were expressed only in infected individuals but not controls.
- A mammalian cell system was used to rapidly create a fusion protein. This protein can then be evaluated using a Luciferase Immunoprecipitation Systems (LIPS) assay. The LIPS assay was then used to compare Ov-16 and other antigens (none of which are expressed in other *Onchocerca*). Sensitivities of up to 90% were identified. With cocktails of antigens sensitivity could be increased to 94-97%, though there remains a small group of people that don't react to any of the antigens.
- Blood from infected Peace Corps volunteers was analyzed to see if any of the antibody responses disappeared over time and could be used as a marker of cure. Some people showed a decline in response over time to some of the

antigens, though seroreversion is not common and seroreversion of none of the antibodies tested appeared to be reliable markers of cure.

- Work has also been done to identify a biomarker of adult female worms. There is a candidate marker that can be measured in sera. NIH is working on getting it into a format that could be used. It might be helpful for mapping in hypoendemic areas and certainly would be useful for the end game. Antigen assays are more difficult to configure than antibody assays so this will take more time and funding.

Key discussion points:

- The sequencing was performed on worms from Cameroon. The proteomics on specimens from Ecuador.
- A test for adult worms would be extremely useful in at least two setting. If development of macrofilariacides is to go forward, an antigen test for adult worms would serve as a measure of cure or reduction in adult worm population in lieu of nodulectomy. It can be difficult to get people to agree to nodulectomy and to find enough nodules to assess the time points needed. The antigen would also serve as a measure of the potential for recrudescence of transmission if IVM is stopped. This could be very valuable in hypoendemic areas or in hyperendemic areas where attempts to accelerate progress to elimination are made, such as vector control or two times a year IVM or doxycycline MDA. The use of Ov-16 as a marker of transmission cannot be used it in these settings because it will give no indication the potential for recrudescence and we cannot assume the adult worms are dead like we can in programs that are 15 years old.
- The specificity of the cocktails of antigens remains quite high because although the background noise accumulates, it is easy to identify a cut point that excludes the noise. There is a risk of additional false positive results because you are using a combination of tests. But as the new antigen appears to be extremely specific, the combination is able to maintain high specificity while increasing the overall sensitivity. A rapid format biplex test with two *Onchocerca* antigens could be created.

Ashley Braman – Mapping Onchocerciasis in Yemen

Key points:

- Yemen has had a long-standing program for providing IVM to people with Sowda, a particular skin manifestation of onchocerciasis.
- Ov-16 ELISA and/or LIPS assays were used to determine prevalence in a group of infected individuals and uninfected individuals for the same communities as the infected individuals.

- 75% of people with Sowda, 35% of people with pruritus but no Sowda, and 28% of people with neither Sowda nor pruritus had positive Ov-16 antibody.
- It appears that the 3 districts with Sowda are mesoendemic and MDA with IVM should be started because 1/3 of the population without Sowda appears to be infected and could serve as a reservoir of infection to maintain transmission under the current strategy.