# Report of the 1st Meeting of the WHO Onchocerciasis Technical Advisory Subgroup

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## I. Abbreviations

| | |
|---|---|
| ALB | albendazole |
| AP | alkaline phosphatase |
| ATP | annual transmission potential |
| CDC | United States Centers for Disease Control and Prevention |
| CI | confidence interval |
| DBS | dried blood spots |
| DfID | United Kingdom's Department for International Development |
| DRC | Democratic Republic of Congo |
| ELISA | enzyme-linked immunosorbent Assay |
| EMRO | Eastern Mediterranean Regional Office |
| ESPEN | Expanded Special Project for the Elimination of Neglected Tropical Diseases |
| EU | evaluation unit |
| FMOH | Federal Ministry of Health |
| FTS | Filariasis Test Strip |
| HRP | horseradish peroxidase |
| iTAS | Integrated transmission assessment survey |
| IVM | ivermectin |
| LF | lymphatic filariasis |
| LGA | local government area |
| MDA | mass drug administration |
| M&E | monitoring and evaluation |
| Mf | microfilariae |
| MOH | Ministry of Health |
| NIH | United States National Institutes of Health |
| NOEC | national onchocerciasis expert committee |
| NTDSC | Neglected Tropical Diseases Support Center |
| OD | optical density |
| OEPA | Onchocerciasis Elimination Program for the Americas |
| OTS | Onchocerciasis Technical Advisory Subgroup |
| PCR | polymerase chain reaction |
| PPES | probability proportional to estimated size |
| Pre-TAS | Pre-Transmission Assessment Survey |
| PSU | primary sampling unit |
| PTS | post-treatment surveillance |
| QA | quality assurance |
| QC | quality control |
| RDT | rapid diagnostic test |
| REMO | Rapid epidemiological mapping of onchocerciasis |
| SD | Standard Diagnostics |
| SSU | secondary sampling unit |
| TAS | Transmission Assessment Survey |
| TFGH | Task Force for Global Health |
| TZ | Transmission zone |

| | |
|---|---|
| UL | upper-limit |
| UOEEAC | Uganda Onchocerciasis Elimination Expert Advisory Committee |
| USAID | United States Agency for International Development |
| WHO | World Health Organization |

## II. Executive Summary

The WHO Onchocerciasis Technical Advisory Subgroup (OTS) was established in order to provide advice to WHO in accordance with the terms of reference developed for the subgroup. The objectives of the 1st meeting were to review current strategies and provide recommendations on potential common strategies or components of common strategies for onchocerciasis elimination mapping, for determining when a stop-MDA evaluation should be performed, for performing stop-MDA evaluations, and to identify key research and operational questions that need to be answered to develop the evidence-base to support strategies for the above mentioned programmatic activities. The key conclusions and recommendations of the OTS are described below. Please note that many of the recommendations are provisional and thus may change over time as new evidence emerges. Evidence that emerges after the meeting will not be reflected in this report. Some lessons will have to be learned while programmes continue to strive to eliminate the transmission of onchocerciasis. Recommendations are based on consensus unless otherwise noted. When consensus could not be reached, operational research questions were defined that should provide the evidence required to obtain a consensus in the future.

1. Serology for onchocerciasis. The OTS recognized the need to standardize Ov16 serology and encouraged the continued collaboration between PATH and the US CDC to evaluate the various formats. Although two versions of the ELISA (one alkaline phosphatase-based and one horseradish peroxidase-based) were selected for continued comparisons, the data presented were insufficient for the OTS to determine that any particular ELISA could not be used for programmatic decisions. Once sufficient data are available that describe the performance of the ELISAs in a variety of epidemiological contexts and in multiple laboratories and once those data are reviewed by OTS, it is expected that the OTS will designate one ELISA as the one for which WHO should support a quality assurance programme. Concerns remain about the sensitivity of the Ov-16 rapid diagnostic test (monoplex or biplex), particularly in low prevalence settings. Because of these concerns, the OTS recommended that dried blood spots be collected when Ov-16 rapid diagnostic tests are used for elimination mapping. If transmission not detected by the rapid test, ELISA would be required to confirm this. If transmission is detected by the rapid test, ELISA would not be required. Rapid test results cannot be used to decide to stop mass drug administration. Finally, development of a new test that could be used to exclude infection in an individual is a priority.

2. Entomology, Vector Monitoring and Control. The OTS recognized that there is an undersupply of the entomology technicians that would be required to perform the various entomological surveys required by the WHO guidelines for stopping mass drug administration and verifying the elimination of human onchocerciasis. It thought efforts should be made to increase country entomological capacity and that the WHO entomological manuals should be updated as appropriate. Updates on progress with traps for black flies and new low-cost techniques for limited vector control were presented. Continue work on both was encouraged, with requests to focus on how to calculate annual transmission potentials when using fly traps and to try the low-cost vector control technique, which consists of training community members to reduce vector habitat, outside Uganda.

3. Onchocerciasis Elimination Mapping. Onchocerciasis elimination mapping is the additional mapping of areas that are not receiving MDA for onchocerciasis but in which transmission is possible. This

additional mapping is required in order to identify all areas with ongoing transmission that need to be treated in order to achieve the interruption of transmission of onchocerciasis. Significant time and effort was devoted to reviewing protocols and data relevant to development of an elimination mapping strategy. General consensus was that the initial strategy should be conservative and biased towards finding transmission. If WHO were to raise the provisional threshold for starting MDA, programmes who started MDA using the lower provisional threshold would not be expected to pass a stop-MDA survey in order to stop treatment in areas that used the lower provisional threshold. A process by which programmes exclude districts that do not need elimination mapping is the first step of the process. For the next step, consensus was obtained that a district-based strategy was an acceptable starting point for mapping and that such a strategy would not preclude more precise determination of transmission zones if needed. Programmes could opt to map by sub-district when the context suggests that transmission is unlikely in the entire district. In areas where transmission is likely and 1st-line villages can be identified, a purposive strategy of village selection is recommended. If the purposive strategy does not identify transmission or if 1st-line villages cannot be identified, then a random strategy of village selection is required. Details of the purposive strategy were agreed upon; for the random strategy additional information is needed though its creation is a priority for future meetings. A provisional threshold for starting MDA was set at 2% Ov-16 seropositivity in adults, as this would bias towards identifying transmission until additional data are obtained. Programmes are encouraged to use the Ov-16 RDT for mapping, with the understanding that results above the provision threshold require starting MDA and results below the threshold require confirmation with Ov-16 of dried blood spots collected at the time the RDTs are performed. This recommendation may change as the performance of ELISA and RDT in low prevalence settings is better described.

*General Outline of the Elimination Mapping Protocol in Areas Not Treated with Ivermectin*

1. Determine areas that may be excluded from mapping.
2. Identify areas where transmission is most likely for the initial elimination mapping and then move out to areas where transmission is less likely
3. Determine the evaluation unit (district or sub-district), this may vary depending on the context of the evaluation
4. Begin with evaluating 3-5 purposively selected 1st-line villages and a minimum of 300 people
    - Use the Ov-16 RDT (countries may opt to use ELISA; either the monoplex or biplex RDT is acceptable)
    - Sample adults ≥ 20 years old
    - If the seroprevalence in a village exceeds 2% then initiate MDA in the evaluation unit
    - If the RDT results are less than the 2% threshold, then they should be confirmed by ELISA
5. If purposive sampling cannot be done or if transmission is not identified by purposive sampling, a random sampling evaluation should be performed
    - Use the Ov-16 RDT (countries may opt to use ELISA; either the monoplex or biplex RDT is acceptable)
    - Sample adults ≥ 20 years old
    - Consensus on the protocol for this was not reached
    - For research purposes, protocols should be designed that enroll people from 30 clusters with an appropriate cluster size to detect an evaluation unit level seroprevalence of 2%

- If the upper bound of the 95% confidence interval of the random sample excludes the tentative threshold of 2%, then MDA is not needed

It should be noted that mapping protocols that are more conservative than those proposed by OTS should be acceptable for decision making at this point in time. For example, if a programme evaluated seroprevalence in children 5-9 years old and found a seroprevalence above the threshold for starting MDA, it would not need to repeat the exercise in adults. However, if seroprevalence in children was below the threshold, mapping in adults would be required.

A number of operational research questions related to onchocerciasis elimination mapping were identified. Some of the key questions are listed here, while all of the questions may be found in the report section of this document. Identification of other environmental factors that exclude the possibility of black fly presence would allow additional districts to be excluded without need for serologic testing. Entomologic studies are needed to help refine the threshold for starting MDA. Studies are needed to determine the minimal number of clusters and minimal cluster size for the random selection of villages component of the mapping strategy.

4. Monitoring and Evaluation. The focus of the discussion of M&E was the creation of a standard approach that would be quick and inexpensive and still provide information to programmes about their progress towards the interruption of transmission. Ideally, routine M&E could also serve as a pre-stop-MDA survey whose results indicate when a programme should proceed with a stop-MDA survey. Citing the experience of many country programmes, the OTS recommended that programmes continue to make use of opportunities to collect M&E information even if they do not align with a defined strategy (e.g. add Ov-16 testing to LF or other NTD evaluations). Routine M&E should continue to use the 1st-line village, using convenience sampling in children aged 5-9 to perform serological evaluations. It was suggested the 100 children in 3 villages per evaluation are would be appropriate. Key operational questions are whether evaluations could be school-based instead of community-based and what the prevalence threshold in the evaluated villages would be that indicate a programme is ready for a stop-MDA survey. It was noted by OTS that coverage surveys and rapid coverage tools can be used in the absence of any diagnostic testing to provide actionable data to programmes. Entomologic M&E was recommended in the WHO guidelines. As it will be important for programmes to know the location of breeding sites, the duration and peak of transmission season, and biting rates, these should be a focus of initial M&E, rather than measuring infectivity of black flies, particularly if the country does not have the laboratory capacity required for poolscreen PCR.

5. Stop-MDA Surveys. Only a few changes were made to recommendations for stop-MDA surveys, though additional changes are to be expected as data become available. One key recommendation is that the minimal sample size for a stop-MDA survey should be 3000 children. There are concerns about not having incorporated test performance, particularly sensitivity, and power to detect seroprevalence below threshold, that are driving this recommendation. Additional recommendations on sample size should be expected as more data become available. It should be noted that a programme that stops MDA based on older criteria and then passes the criteria for the post-treatment surveillance period would not be expected to repeat the stop-MDA survey in order to meet the new criteria. The second key recommendation is that only children ages 5-9 years old should be included. This was based on concerns that testing younger children provided little information on the

status of transmission and provided false assurance that transmission has been interrupted. There is interest in changing the threshold for stopping MDA, and there is some modelling data to suggest that this may be feasible. However, field data are needed, so it was recommended that operational research studies evaluate a provisional serologic threshold of 1% for stopping MDA. It should be a priority to use vector infectivity prevalence surveys to provide insight about potential changes in the threshold for stopping MDA. There was much discussion about the need for an evaluation of 1st-line villages and a random evaluation of other villages before deciding to stop MDA. As the data needed to make any decisions about this were not available at the meeting, it was recommended that comparisons of the 1st-line village and random sampling approach be prioritized.

**Conclusion**

The transition from the goal of control (or elimination as a public health problem) of onchocerciasis to the interruption of transmission of onchocerciasis necessitates a reappraisal of the methods used to evaluate programme progress and success. Although this change is important and exciting, it does require the development of some common approaches and the willingness to continue to adapt these approaches as new data become available. The executive summary contains the highlights of discussions and recommendations made during the 1st meeting of the OTS. More details are contained in the body of the report. The OTS will continue to meet on a regular basis to review strategies and new data as they become available so that we can continue to address the needs of programmes as they begin the push to elimination.

## 1. Introduction

Despite the release of the 2016 WHO guidelines for stopping mass drug administration and verification of the elimination of human onchocerciasis, many challenges remain for implementing the guidelines. Additionally, many country programmes are transitioning from disease control to interruption of transmission. In order to augment the guidelines with common strategies for a variety of programme activities needed to achieve elimination and to facilitate the development of the evidence-base required for development of new guidelines, the Onchocerciasis Technical Advisory Subgroup (OTS) was established. The OTS provides advice to WHO in accordance with the terms of reference developed for the subgroup. The objectives of the 1st meeting were to review current strategies and provide recommendations on potential common strategies or components of common strategies for onchocerciasis elimination mapping, for determining when a stop-MDA evaluation should be performed, for performing stop-MDA evaluations, and to identify key research and operational questions that need to be answered to develop the evidence-base to support strategies for the above mentioned programmatic activities.

Regional updates were presented by representatives of the Expanded Special Project for the Elimination of Neglected Tropical Diseases (ESPEN) and the Eastern Mediterranean Regional Office (EMRO). Despite successes with scale-up of treatment and, in limited areas, scale-down of treatment many challenges were highlighted. The major challenges included: the need for a strategy to complete elimination mapping, new strategies for the accelerating the interruption of transmission, insufficient entomological and laboratory capacity, confusion about which Ov-16 serologic test to use for evaluations, financial support for some areas that need treatment, and political instability and security issues.

## 2. Serology for Onchocerciasis

Presentations:

Although the 2016 WHO guidelines specify that Ov-16 serology must be used to determine seroprevalence of the antibody in children younger than 10 years old as part of the evaluation required for stopping mass drug administration (MDA), no recommendation is made in regards to a specific assay other than to state that the rapid diagnostic test (RDT) format should be validated prior to using that format to make stopping decisions. Currently multiple ELISAs are in use but there is little published evidence on the performance characteristics of the existing assays.

CDC and PATH are currently working to develop a standardized Ov-16 ELISA protocol, with support from the Task Force for Global Health. An initial comparison at CDC focused on four ELISAs, protocols, two of which use an alkaline phosphatase (AP)-based enzyme and two of which use a horseradish peroxidase (HRP)-based enzyme. The AP ELISAs were the version used by the Onchocerciasis Elimination Program for the Americas (OEPA) and the protocol used at CDC. The HRP protocols were from PATH/Smith College and a prototype kit developed by Standard Diagnostics (SD). The results of the comparisons were broadly similar, so additional aspects of each test were considered, including anticipated availability of reagents and controls, potential assay variability due to temperature change and expected throughput, in order to move forward with comparisons. Two protocols (CDC-ELISA and SD-ELISA) were compared in more detail. Serum samples collected from *O. volvulus*-endemic areas in Africa and negative samples from various non-endemic settings were used for the comparisons. Three different methodologies were used to determine potential cut points for distinguishing positive and negative results. Some of the cut points resulted in sensitivity near 80% but the specificity was unacceptably low. For those cut points that resulted in a near 100% specificity, the sensitivity of the two ELISAs was around 60%. PATH performed its own analysis of an updated version SD-ELISA that was different than the version that CDC evaluated with a different set of samples; the positive samples were taken from Guatemala, Ecuador, and Uganda. This analysis determined the SD-ELISA to have a sensitivity of 70–80%. It is important to note that the prototype of the SD-ELISA evaluated in the PATH presentation was a new version that had been reformulated because of the results of the above mentioned CDC analyses. The cut point of the two ELISAs had not been finalized, so final performance characteristics are not available. The new prototype of the SD-ELISA kit evaluated by PATH should become available for research use in the 1st quarter of 2018. Programmatic considerations that will need to be taken into account if both ELISAs prove to be equivalent include the cost of the CDC-ELISA compared to the SD-ELISA kit and the throughput time of both ELISAs.

A technical issue was identified when evaluating the SD ELISA with dried blood spots (DBS). Elevated background noise was observed when testing DBS from non-endemic areas. Various blocking steps are being explored to help reduce the non-specific signals. Although the results were not available at the meeting, this issue appears to have been resolved. Data will be presented at a future meeting of the OTS.

Once the ELISA protocols are optimized for DBS as the sample type, a large number of DBS that were collected during field evaluations of the RDT can be analysed in order to compare the field

performance of the Ov-16 RDT to Ov-16 ELISA results. CDC compared the performance of the RDT to the ELISA in a people who lived in Muheza district, Tanzania. The district is co-endemic for onchocerciasis and LF and had implemented at least 10 rounds of MDA with ivermectin. The Ov-16 RDT was compared to the Smith HRP ELISA. This limited comparison confirmed the previously identified concerns about low sensitivity of the RDT when compared to ELISA. In all age groups (5–9, 10–15, 16–19, 20–29, 30–39, 40–49, and ≥ 50 years old), the ELISA identified more individuals as positive. On average, the ELISA identified twice as many people as positive compared to the RDT. Preliminary analyses comparing ELISA optical density (OD) results to RDT results did not reveal a pattern explaining the difference in test results; there were individuals with high OD readings by ELISA who had negative results by RDT. A larger analysis will be important.

A brief progress report was given on the status of the assessment of laboratory capacity for the African programmes that will need to be able to perform both Ov-16 ELISA and the O-150 polymerase chain reaction (PCR) of blackflies. Six laboratories have been assessed and three more laboratories will be assessed soon. Assessments, which are supported by the United States Agency for International Development (USAID) and United Kingdom's Department for International Development (DfID) include an evaluation of the equipment possessed by the laboratory, the frequency at which it performs similar tests, and the personnel available for performance of testing. The initial intention is to establish a regional network of laboratories that can begin to perform the needed testing for elimination mapping and/or stop-MDA surveys and can support other country laboratories as they begin performing testing as well.

Discussion: Serology

The discussion of the presentations focused on several themes. The first was that although the ELISA standardization is still in progress and the final cut point for determining whether the test result is negative or positive has not be agreed upon, the comparison so far has shown that the available ELISAs perform similarly, although the performance evaluations are dependent on which specimens were tested and the laboratory settings were ideal and not necessarily representative of performance in field laboratories. Two ELISAs that seem to be better options for use by programmes are in the final stage of comparison, which will include a focus on personnel-time and cost in addition to test performance. The test performance comparison should take place in multiple labs and include specimens from a variety of sources, including low transmission areas. An evaluation of repeatability/reproducibility between labs will be an important component of comparison. Evaluation of the ELISA should take place in at least one laboratory in Africa. More robust cost analyses are needed that include workflow and hidden costs. Finally, the issue with DBS should be resolved in the next few months. It will be necessary to develop standard operating procedures both for how to use the DBS with ELISA and how to maintain the DBS from the time of collection until the time of analysis. DBS requires both a cold-chain and the use of desiccants.

Although a more in-depth comparison of the RDT to the ELISA is needed, initial evaluations continue to show a decreased sensitivity of the RDT compared to ELISA in low prevalence settings. The RDT detects a prevalence that is about 50% less than the ELISA. The biggest difference was seen in the 5-9 year old age group, where the ELISA found a prevalence of around 4% and the RDT found a prevalence of 0.1%. As the discordant results between ELISA and RDT include samples with both

borderline and high OD readings, it cannot be concluded that RDTs are failing to capture only low-titer infections. Because of the uncertainty of the performance of the RDT in such settings, it is still not possible to recommend the use of the RDT alone during stop-MDA surveys. It should be acceptable to use the RDT for routine M&E and for mapping. However, until the performance of the RDT is better understood, programmes should continue to collect DBS during elimination mapping exercises in order to confirm results in areas where no infection is detected.

Quality assurance (QA) will be key moving forward because country programmes and their expert committees will be basing their decisions on laboratory test results. WHO and other groups already exploring options on how best to create a QA panel and associated training that could then be shared with laboratories. Finalization of a QA process will have to wait until an ELISA is selected.

OTS Recommendations: Serology

1. CDC and PATH are encouraged to continue to collaborate on the comparison of the ELISA protocols. Ideally specimens from the ESPEN lab, which would have accompanying Ov-16 RDT and skin snip results, should be used to compare the two ELISAs in multiple laboratories.
2. CDC and PATH are encouraged to collaborate to determine the appropriate cut point for the SD-ELISA.
3. CDC is encouraged to share specimens that have been collected for the CDC serum bank. There are samples from both minimally treated hyperendemic areas and formerly hyperendemic areas where transmission has been suppressed by ivermectin treatment.
4. When reaching a decision on which of the two ELISAs should be recommended for programmatic use, it will be important to consider sensitivity, specificity, cost, throughput, and performance in Africa-based laboratories.
5. It is not possible to recommend one version of the ELISA over any other at the present time. However, as the performance of the ELISAs were comparable, laboratories should continue using the ELISA that they have experience with while waiting for the selection of which ELISA should be supported. There needs to be a system of quality assurance established for each of these laboratories.
6. Once an ELISA is selected as the assay platform of choice by WHO, WHO will need to ensure that a system of quality assurance is established to ensure that country programmes are basing their decisions on high quality laboratory data.
7. At this time the Ov-16 RDT still cannot be used for stop-MDA surveys. It may be used for elimination mapping, though programmes should collect DBS at the same time that they perform RDT testing. If the RDT results suggest an absence of transmission, this will need to be confirmed by ELISA. If the RDT results suggest the presence of transmission, no additional testing would be required.
8. A confirmatory test, particularly one that can be used to exclude infection is a priority.

**3. Updates on vector monitoring and control**

Presentation: Brief overview of vector surveys: The overview was given to help inform decisions of the committee. Vector surveys help determine the geographical area of transmission, help define transmission dynamics, help select 1st-line villages for M&E and other epidemiological assessment, are required for PTS and stop-MDA surveys, and help understand the potential for recrudescence after MDA is stopped. A lot is already understood about how best to design vector surveys, though concepts such as seasonal variation of the location of breeding sites may need to be updated. Even if breeding sites are not fully evaluated prior to elimination mapping, eventually they will need to be evaluated. Even in the absence of PCR capacity, vector collections can provide a lot of useful information such as parity-rates, annual biting rates, determination vector species (and thus their flight ranges), etc. High biting rates in newly identified areas could influence programmes' initial elimination strategies.

OTS Discussion and Recommendations: Brief overview of vector surveys

The discussion of the brief overview began with the fact that it may not always be easy to identify breeding sites and traditional methods may miss some breeding sites, so more work is needed to determine how best to identify breeding sites that contribute to transmission in low transmission areas. It was known at the time of the development of the REMO approach that the strategy for identifying 1st-line villages would work best in savanna areas and that additional methods might be needed for forest areas and definitely were needed for areas where *Similium neavei* was the vector. Strategies specific for low transmission areas may require additional operational research that could be performed as mapping progresses. Much of the vector work will be challenged by an undersupply of entomology technicians who can go into the field and verify breeding sites, oversee fly catches, assist with vector studies, etc. Efforts will be needed to increase black fly entomological capacity in onchocerciasis-endemic countries. Updating the vector manuals should be a priority for 2018. Programmes also need to update their understanding of the dynamics of their breeding sites. Transmission seasons may have changed over time due to changes in the timing of the rainy season, and breeding site locations may have changed as well.

Presentation: Vector monitoring and control. Work continues on the Esperanza Window Trap. The performance has varied from one setting to another. In Mexico, where it was initially developed, the trap collected 50-75% as many flies as a human landing capture team when operated by trained entomologist and 25% as many flies when operated by community members. In Burkina Faso the trap collected 100-150% as many flies. In Uganda the traps collected on average more than 150% as many flies as human landing capture teams. Modelling may allow the capture by the traps to be used to calculate an annual transmission potential. The trap performance would have to be evaluated and calibrated in a variety of settings.

Promising results have also been obtained recently on a new approach for vector control, called 'slash and clear', that involves the use of community members to reduce vector breeding habitats near afflicted villages. Preliminary trials suggest that this approach can result in dramatic and relatively long lasting suppression of vector biting in afflicted villages. Ongoing studies are planned

to examine long-term effectiveness and sustainability. Modelling may help to determine potential impact of this method on the projected time to elimination of transmission.

Discussion: Updates on vector monitoring and control

It was pointed out that trap placement can affect the capture rate (e.g. traps within 30 meters of each other can have markedly different capture rates), so explicit guidance on how to position traps in order to optimize the catch. It was also suggested that a substitute for tanglefoot, which is the glue used to trap the flies, be sought because it can be difficult to ship the chemical overseas due to its classification as a 'hazardous good'. It was also noted that non-Similium flies are caught (including the *Glossina fuscipes* and *G. pallidipes* vector species for human African trypanosomiasis). The various flies should be identified to determine if the traps could be used for vectors of other fly-transmitted diseases.

There was also a discussion about the potential durability of the slash and clear methodology of vector control. It is possible that the blackflies could adapt and simply create breeding sites elsewhere. This will need to be monitored and strategies developed to maintain the impact of the methodology.

OTS Recommendations: Vector monitoring and control

1. Continued work on the traps was encouraged, with a focus creating a system for determining the annual transmission potential in a variety of ecological settings.
2. Continue work on the community-directed vector control was also encouraged. Its utility in different contexts should be examined, as it has only been evaluated in Uganda.

**4. Elimination Mapping for Onchocerciasis**

i.Presentation: Ethiopia's Mapping Protocol

Ethiopia created a mapping protocol because it decided to move from the control phase to the elimination phase. Neither the programme nor the national onchocerciasis expert committee (NOEC) felt that nodule prevalence was a suitable indicator for elimination. In collaboration with its NOEC, the programme designed a mapping protocol, which is described in national documents. Elimination mapping has been implemented in some of the 300 districts (woredas) that have not been assessed previously for onchocerciasis. No results were available at the time of the meeting.

The criteria for starting MDA in an untreated district area are:
i) ≥1% of 300 residents 5-10 years old test positive with Ov-16 ELISA
or
ii) >10% of 30 residents ≥20 years old test positive with Ov-16 ELISA

All previously unmapped districts are to be mapped. The first step of the mapping process is to use topographical maps to find areas with rivers and elevation changes that may signal potential breeding sites. Villages that are near rivers are identified and villages within 10 km of potential breeding sites are designated as 1st-line villages. Up to 3 communities are selected in the district for evaluation.

In each community, 10 adults ≥20 years old and 100 children 5-10 years old are randomly selected for testing with Ov-16 ELISA. Assuming three communities per district, this results in a total sample size of 330 per district. Ov-16 RDTs will be incorporated when available and if appropriate. If in that total sample the Ov-16 ELISA test shows ≥1% positive children or ≥10% positive adults, then the district will be classified as endemic. Given the sample size and the decision to statistically exclude 1% and 10%, a single positive adult or child will exceed the threshold because the confidence interval around the estimate would include the threshold.

The highest priority areas for mapping are those that are on the edge of known treatment areas. A desk-based spatial analysis is also planned.

Discussion: Various aspects of sampling were discussed. It was suggested that if all of the identified 1st-line villages are deemed equally at risk, geographic distribution could be taken into account. Concerns were expressed about determining that a village is a 1st-line village without verifying the presence of a breeding site. However, it may be difficult for many programmes to rapidly verify potential breeding sites particularly given the limited number of entomologists and field entomology workers with expertise in black flies.

There was agreement that Ethiopia was erring on the side of caution, both in proposing to assess areas that would not have been assessed under the previous control paradigm and in proposing a low threshold for the initiation of treatment. The question was raised as to whether the vector is in fact present in the large sections of the country where mapping is planned; it was agreed that vector presence should be considered as part of the decision where to map (see exclusion mapping).

Concern was also raised about the district oriented approach to mapping, which appears to be an attempt to force methodology used by the lymphatic filariasis programme to work for onchocerciasis. Although the mapping was to be district-based, the focus is still on 1st-line villages in association with breeding sites and the eventual delineation of the transmission area. However, using a district-based approach allows programmes to begin to define transmission in an area using the administrative structures already in place.

After concerns were express about whether all districts in Ethiopia should be mapped in order to have that information for its verification dossier when the time came to submit the dossier to WHO, WHO clarified that there is no expectation that mapping with serology is needed in districts in which there is no reason to expect to find onchocerciasis transmission (e.g. the environmental conditions would not support black flies or there is a known absence of black flies). It would, however, be important to document the approach used to exclude areas from mapping.

ii. Presentation: Ghana Elimination Mapping Study Protocol

The Ghana protocol was more of a rapid assessment of transmission throughout the country. However, it included assessment of treatment naive areas. The programme evaluated 161 districts (across 7 river basin-based transmission zones), including 50 hypo-endemic districts with no history of MDA, to determine what treatment level if any was required. Seven non-endemic control districts were included in the 161 districts.

1st-line villages, defined as villages within 5 km of a breeding site, were evaluated with purposive sampling of two 1st line communities in each selected district; occasionally a 3rd 1st-line village was evaluated in a combined evaluation of >1 district because of the locations of the breeding sites/river in the area. In the control (non-endemic) districts 2-3 villages were selected. Large urbanized communities were excluded from the evaluation. In total, 312 1st-line villages and 15 control villages were evaluated.

In each village, 75 children <10 years, selected by purposive sampling, were tested with Ov-16 RDT; if fewer than 75 children were present, then all children were enrolled. DBS were also collected from i) all children testing positive by Ov-16 RDT, and ii) a 10% systematic sample of the children tested by Ov-16 RDT, for subsequent laboratory analysis with ELISA, both as a quality control and to compare with sensitivity and specificity of the RDT (ELISA results were not available at the time of the meeting). In each village 300 adults ≥20 years were purposively selected and tested with skin snip microscopy as part of the transition between types of diagnostic test. If fewer than 300 adults were present in the village, then all adults were enrolled. Black flies were collected for O-150 PCR and poolscreen analysis, but results were not available at the time of the meeting. The threshold of Ov-16 RDT-positives to consider a district for MDA was not specified in the protocol, but it was assumed that >0.1% at the one-sided UL of the 95% confidence interval was used. For skin snip microscopy, Mf prevalence of 0%-<1% would result in endemic districts continuing to receive MDA at the same frequency; prevalence of ≥1%-<5% would result in consideration for annual MDA; and prevalence ≥5% would result in consideration for twice-yearly MDA.

The surveys found prevalence to be lower in some areas than thought and higher in others. Of particular note was that several areas where no positive skin snips were found had >1% and sometimes >5% seropositivity by Ov-16 RDT. Multiple untreated hypo-endemic districts were found to need MDA for onchocerciasis. Some of the 7 river basins need to be examined in more detail because the snip and RDT data suggest that they may be broken into smaller transmission areas, and stop-MDA surveys would need to be performed in some of the smaller areas; the country, possibly with the input of its NOEC, will need to review the available information to determine if it makes since to do this.

Discussion: It was agreed that although it was clear that the Ghana protocol was not designed purely as a mapping exercise, the results obtained were useful. They clearly demonstrated that skin snip microscopy results may suggest that transmission has been interrupted while serology detects ongoing exposure in children. The data also suggested that serology might be able to be used to break large transmission zones into smaller areas. It would, of course, require the expertise of the country programme to determine if it makes sense to break a large zone into smaller areas for evaluation and stop-MDA decisions. The need for a standardized elimination mapping approach across countries was recognized, even in the absence of data confirming that the best approach has been identified. It was also noted that the sentinel village approach is limited to identifying district in which transmission is occurring; it does not define the boundaries of transmission. The lack of a fully described mapping protocol, however, did not prevent Ghana from identifying a large number of untreated hypoendemic districts that require MDA. Some districts may need to be revisited to better describe sub-districts that require MDA, and elimination mapping will likely be required in areas that have not been identified as needing treatment. There was general agreement that until the ELISA comparison to RDT is complete, it remains unclear whether RDT alone can be used for elimination mapping, but its use can help programmes prioritize scale-up of MDA in those areas with a detectable signal of ongoing transmission with the RDT.

iii. Presentation: Two-Phase Approach to Onchocerciasis Elimination Mapping

Whereas the traditional approach to mapping onchocerciasis called for purposeful sampling in the areas of highest risk, mapping with the goal of elimination will be performed in any unmapped areas where transmission is possible, including those where the level of risk of on-going transmission is not well known. In addition, greater certainty is required to declare an area suspected of having transmission as non-endemic. It is important that the approach should provide findings at an administrative-unit level (e.g. district) to align with the level at which interventions will be administered.

A two-phase approach to mapping could fulfill these requirements. Phase 1 involves looking for signs of transmission in the most likely places, using a sensitive approach to include all areas clearly in need of MDA. This should be simple, quick, and biased in favor of finding onchocerciasis and initiating MDA. Phase 2 seeks to exclude the possibility of transmission in the survey area, using broad, representative sampling to ensure any ongoing transmission is not missed. A rigorous approach would add confidence in the decision to classify areas as non-endemic.

In Phase 1, the assessment area is the district and the primary sampling unit (PSU) consists of 1st line villages, with 4 villages selected using purposive sampling based. Villages with the highest likelihood of transmission are selected based on known indicators of risk. The secondary sampling unit (SSU) consists of all residents ages ≥10 years, with a convenience sample of 300 people per 1st line village. Older individuals are selected to bias towards starting MDA. The test to be used is the Ov-16 ELISA because the RDT is not yet an option, given current challenges. A result of >X% will trigger district-wide MDA, while a result of <X% will propel the district to Phase 2. The threshold 'X' will need to be defined by the committee. (This was particular protocol was presented to generate discussion; see (XXX) for the recommended protocol).

Phase 2 consists of a cluster survey within the district, taking schools as the PSU as this will make the survey easier to implement; given that the entire district is targeted, it is fine if the students come from different communities. The sample is 30 schools, selected using PPES, which is advantageous as it keeps the sample size constant within each school and allows easier planning by the survey team. The SSU is students 10-14 years old, as prevalence tends to be greater in older children, with 100 students per school selected via a systematic sample (lining up eligible students and applying a sampling fraction to get ~100 students in the target age-group). A result of >X% positives overall will trigger district-wide MDA, while a result of ≤X% will classify the district as non-endemic. (This was particular protocol was presented to generate discussion; see pages 25-26 for the recommended protocol).

Discussion: It was agreed that the mapping strategy should proceed, starting with priority areas where evidence suggests that transmission is possible. A provisional strategy would be acceptable, especially given the urgency to initiate the process and the need to learn as we advance with mapping from areas of higher risk to areas of lower risk. As the earlier districts complete elimination mapping, lessons learned or new indicators identified during the elimination mapping within a subset of districts could potentially be useful for excluding other areas from the elimination mapping process (e.g. identification of a new indicator that indicated that black flies could not be present).

Complementary approaches to help identify districts for mapping were also discussed, focusing on identifying the presence of the vector. One idea was to use satellite imaging coupled with the ESPEN database to establish ecological limits. It was noted that the cost of the former could be an obstacle, with the cheapest being RapidEye images (with resolution of 3-5 meters) at about $1.28/km$^2$; LandSat imagery (10-15 meters) was deemed insufficiently detailed to be able to identify key areas. If satellite imaging could be used to identify potential breeding sites for *S. damnosum* and to exclude some areas from needing elimination mapping, some physical verification of the presence or absence of breeding sites would be required in order to confirm the satellite findings; this would likely need to occur in a variety of ecological settings.

The district versus non-district-based approach is one that engendered significant discussion. Some felt that delineation of transmission zones should be part of the mapping strategy, unless the idea is to treat the entire district regardless of whether transmission occurs in the entire district. Many felt that the district approach made sense and did not exclude the possibility of definition of transmission zones. A strategy for initial roll out of elimination mapping that targets higher risk areas first (e.g. the edge of known hyper- and meso-endemic treatment areas) would allow

programmes to start MDA rapidly in these areas where the fine details of the strategy for the lower risk areas (e.g. in areas where the environment is unfavourable towards supporting black fly populations) are determined later. The The two-phased elimination mapping approach could be similarly applied in a district or sub-district, so long as the administrative unit chosen aligns with the administrative unit that will be used to deliver the intervention. It is important to keep in mind that there is a significant cost component associated with the choice of survey and treatment area.

And important discussion was had about of the cost of elimination mapping. Although efforts should be made to minimize the cost of mapping, otherwise one would just perform a stop-MDA survey in all potentially endemic areas and then start MDA anywhere that failed to pass the survey, it is important to remember that *elimination* mapping requires more rigor than required where the target is control. A more expensive strategy that correctly identifies all places in need of treatment should be preferred over a less expensive strategy that misses areas of transmission.

There was also discussion about the appropriate age group to use for the mapping strategy. The OTS asked the presenter to prepare slides for the next day to review what was available to answer this question (see the section on Ov16 prevalence by age in low transmission areas).

OTS Recommendations:

1. A district-based (or sub-district-based) mapping strategy is acceptable as the starting point for elimination mapping.
2. Use of satellite imagery to assist elimination mapping, particularly in terms of excluding areas that might not require elimination mapping, should be explored.
3. The mapping strategy should be biased towards finding transmission and thus, in general, older age groups are preferred for mapping as opposed to evaluating children ages 5-9 years old.

iv. Presentation: Ov16 prevalence by age in low transmission areas

For elimination mapping it is important to bias the evaluation towards finding transmission. If an age group is selected that is too young, a signal of transmission may be missed. Ov-16 RDT results by age from surveys conducted in three countries—Gabon, the Democratic Republic of Congo (DRC), and Cameroon—in hypo-endemic areas were presented. For Gabon and DRC, results were disaggregated by age group: 5-9 years, 10-14 years, 15-19 years, 20-29 years, 30-49 years, and ≥50 years. For Cameroon, the age groups were the same apart from the 5-9 years group which was not included.

In Gabon the age group results were presented from 67 purposively-selected villages in an area that had not undergone MDA. A total of 4222 people was tested with the Ov-16 monoplex RDT. Prevalence in the 5-9-year-old age group was a little more than 1%. Prevalence among 10-14-year-olds was more than triple that of 5-9-year-olds and was close to the prevalence among the adult age groups.

In DRC 799 people were tested with the Ov-16 monoplex RDT in an area that had not undergone MDA. Prevalence was around 2% in the 5-9 and 10-14-year-old age groups. Prevalence increased 4-fold in the next oldest age group in did not peak until the 30-49-year-old age group.

In Cameroon 3389 people were tested using the Ov-16/Wb-123 biplex RDT in an area that had not implemented MDA with ivermectin. Prevalence in the 10-14-years-old age group was less than 2%. It was slightly higher in the next two age groups and 4-fold higher in the 30-49-year-old age group.

Across the three countries, it was observed that in untreated areas prevalence tended to increase by age. In the two countries where 5-9-year-olds were tested, signs of transmission were apparent in that age group; this was not indicative of prevalence in adults, however, which were better represented by the 10-14 years age-group.

Discussion: It was discussed that the rate of increase in sero-prevalence by age is an important consideration, and it was not unexpected that in low transmission areas the rise would occur more slowly. There can be a fair amount of variability due to local transmission dynamics. For example, in hyperendemic areas in Uganda, Ethiopia, and the Democratic Republic of Congo, all of which had received 3 or fewer rounds on MDA, each area had a different rate of increase, although up to 30% of children under ten years of age were positive in one site. In one site, seroprevalence reached the overall adult seroprevalence by the 10-15-year-old age group. In others it took 5-15 years longer to achieve the overall adult seroprevalence. It was proposed that adults over the age of 20 years be used for mapping in order to insure that the signal of transmission was not missed. Concern was expressed that antibody reactivity does not mean current infection, so it was possible that antibody positivity in adults would not be a good indicator of transmission. However, in untreated areas with active transmission it is not clear that this is a valid concern as most adults would be expected to still harbour active infections. There was consensus that the age group evaluated should be older than the age group used for stop-MDA evaluations. The 15-19 years or older more generally approximate the overall adult seroprevalence, but 15-19 year olds can be hard to located for evaluation so the debate centered over the 10-14 year olds and the ≥ 20 year olds. The use of children was previously thought to be a surrogate for incidence, and it was also thought to be most feasible. The group recognized that older age-groups are in fact more sensitive indicators. An age-group of ≥20 years was recommended for elimination mapping, as this is more likely to show a signal than younger age-groups. Males and females are both to be tested, though it is still generally thought that males are at greater risk.

OTS Recommendations:

- The age group evaluated as part of elimination mapping should be composed of individuals ≥20 years old.
- Operational research comparing the 10-14-year-old age group and the ≥20-year-old age group should be a priority.

## 5. Overall Discussion of Elimination Mapping

There was extensive discussion aimed at deciding on a strategy for elimination mapping. The group agreed that the strategy could consist of four main steps:

1. Exclusion: Determine those areas that do not need elimination mapping
    a. Look at ecological and habitat classifications, perhaps using remote sensing, to identify zones where onchocerciasis never existed and/or where there is limited possibility it is present.
2. Classify the remaining untreated areas into levels of risk for prioritization: transmission likely or transmission unknown
    a. Likely areas would include those that border hyper- and meso-endemic areas and those with known disease or black fly nuisance.
3. Purposive sampling of high risk villages in the district or sub-district
    a. Typically these are 1st-line villages
4. Random sampling of the rest of the district or sub-district when purposive sampling does not find evidence of transmission

<u>i. Exclusion Mapping</u>:

Exclusion mapping has been conducted successfully for several other diseases, including malaria and trachoma. The general approach, which is used to exclude areas for control interventions, consists of collecting and compiling available data, overlaying those data with environmental features, analysing the combined data, and following up with confirmatory surveys where needed. This approach is now relevant for onchocerciasis in the framework of elimination because prior rapid epidemiological mapping of onchocerciasis (REMO) for delineation of treatment areas under APOC, while extensive, may have missed areas of transmission where there were few people with symptoms. Significant work has already been completed by ESPEN to identify districts that may need elimination mapping for onchocerciasis and it has categorized these districts as those receiving MDA for lymphatic filariasis and those not receiving any ivermectin MDA. It is working to identify criteria by which to exclude districts from requiring elimination mapping because transmission would be deemed to be not feasible.

The broad exclusionary process conducted by ESPEN began with a total potential list of 4800 districts that are not under treatment for LF or onchocerciasis and that have not been assessed for onchocerciasis. 3225 districts were excluded from this list based on presumed unsuitability of habitat (climate, temperature, dryness), resulting in a list of 1575 districts potentially in need of elimination mapping. Although further exclusion of districts can likely be done and this list likely contains districts that do not need elimination mapping, the group agreed that this list was a good start and the it should be used in combination with other available datasets and local country knowledge to develop a more definitive list of districts requiring elimination mapping for each country. It was recognized that the status of districts was susceptible to change over time (e.g. REMO was completed many years to decades ago in some areas) so districts included in the REMO process should be reviewed again. Districts that are receiving MDA for LF are not a priority for elimination mapping because they are already receiving ivermectin. However, evaluations of onchocerciasis transmission should be planned to coincide with the LF programme's next evaluation (e.g. transmission assessment survey).

The process of overlaying REMO and other data with environmental factors was discussed. It was noted that in the past, high-resolution maps (commonly used under OCP) showed features such as marshes (where *S. damnosum* would not be present) and man-made features, such as dams. These maps, particularly those of 1:250,000 scale if available, could be further interrogated to help exclude areas under the elimination paradigm.

Satellite imagery has the potential to be used for exclusion mapping, though high resolution maps can be expensive to access. As exclusion mapping aims to identify where breeding sites are not present, rather than where they are, lower resolution mapping imagery may be sufficient. As discussed earlier, the use of satellite imagery to exclude (or confirm) the presence of breeding sites may require some validation. In those locations where validation of vector absence or presence is deemed necessary, test collections to confirm the presence of *O. volvulus* vectors would be sufficient.

During the discussion it was clarified that the OTS's role was to review the available methods for exclusion mapping and encourage the development of a common strategy. It has no role in determining which specific districts need or do not elimination mapping. That decision is a country decision. Countries may want to discuss their decisions with their NOEC and ESPEN. It may be necessary for face-to-face meetings between ESPEN and countries to finalize the exclusion process.

The group agreed on the importance of identifying the minimal and sufficient information to justify conducting a mapping survey. Cost-effectiveness will be an additional factor to consider, to be balanced with support for the activities needed to achieve the elimination goal. The group agreed that mapping should not automatically be conducted everywhere as this would result in wasted funds; a strategy should be developed, iteratively and with learning at each step, to more clearly identify where mapping surveys are required and where they can be excluded.

<u>OTS Recommendations for exclusion mapping</u>:

1. Exclusion mapping should be part of the standard elimination mapping methodology.
2. The methodology used by ESPEN to establish its existing list should be clearly documented and provided to the OTS
3. ESPEN should ensure that the list of districts that need to be reviewed for elimination mapping has been shared with country programmes and that the opportunity to refine the list with input from ESPEN, if desired, is made available to the programmes. A final list documenting which districts need elimination mapping after country review should be created.
4. An operational research priority would be the development of a predictive model that incorporates map imagery and other environmental data

<u>ii. Discussion: Purposive Sampling</u>:

Purposive sampling requires identification of areas of high risk for transmission, which is primarily the identification of 1st-line villages, which are those villages closest to vector breeding sites. Ideally, the presence of breeding sites would be verified by visiting the breeding sites and collecting limited numbers of flies. However, given the scale of purposive mapping this may not always be possible. It may be acceptable simply to identify villages that report black fly nuisance and the presence of

rapids nearby. Asking villagers and/or district health officers about black fly nuisance and the presence of rapids would be the 1st step of more formal breeding site investigations, so it was agreed that this should always be done. Good contour maps may be helpful in identifying rapids. The group was undecided on whether determining the presence/absence of the vector was sufficient, or whether it was also necessary to identify breeding sites. It was agreed that an advantage of identifying breeding sites is that it would then be possible to establish a flight-range radius for the vector. At the same time, it was acknowledged that focusing on village proximity to river and areas of rapid water-flow may not be sufficient for identification of areas requiring treatment and/or exclusion of areas from the need for mapping (e.g. results were presented from operational research studies which found high levels of infection in villages that were not close to known breeding sites).

The group agreed on the importance of identifying the minimal and sufficient criteria (some combination of entomological and epidemiological markers) to justifying conducting a mapping survey. Cost-effectiveness will be an additional factor to consider, to be balanced with support for the activities needed to achieve the elimination goal. The group agreed that mapping should not automatically be conducted everywhere as this would result in wasted funds. New criteria for excluding districts or sub-districts from elimination mapping may be learned as part of the implementation of the elimination mapping surveys. These criteria should be disseminated rapidly once identified.

Some frustration was expressed about uncertainty about components of the mapping strategy (discussed at this stage and later stages). It was agreed that in the higher risk areas (e.g. adjacent to treated hyper- and meso-endemic areas or in areas with known presence of black flies) it is likely that identification of breeding sites and 1st line villages will be more straightforward and mapping will proceed quickly. As mapping moves into lower risk areas, strategies may need to be adjusted and in some cases even micromapping (e.g. village level mapping) may be required. It was acknowledged that some components of the overall mapping strategy will have to be developed as experience is gained.

It was pointed out that lessons could be learned from the experience of mapping for LF. LF mapping based treatment decisions on the results from two purposefully selected sites, with one or more positive individuals triggering MDA. Variations in how these two sites were chosen and the meaning of a single positive result have, in some instances, led to challenges for the program. This experience suggests that an investment in a more complex strategy now could pay off in terms of resources or time to elimination compared to a simpler more rapid strategy now.

iii. Random Sampling:

The need for random sampling was discussed quite a bit. One group argued that the 1st-line village approach should be used even in the lower risk areas. If it was more difficult to identify the breeding sites in these areas, more effort should be dedicated to identifying the breeding sites. The other group argued that recent experiences (such as finding villages that had high prevalence of Ov16 positivity in DRC that were neither the village closest to the river nor closest to known breeding sites) indicate that we still do not fully understand how to identify all the breeding sites that contribute to transmission. Therefore a random sampling methodology needed to be identified for use in areas where either the 1st-line village approach had failed to identify transmission or areas where the

location of breeding sites was uncertain. OTS requested that one member of the committee present on findings from studies of the co-evaluation of onchocerciasis and lymphatic filariasis (see page 32, presentation on a comparison of purposively selected first-line villages vs. randomly selected villages) to better inform the discussion. In brief, the study identified non-1st-line villages that had higher prevalence after years of MDA than known 1st-line villages, supporting the concept that random sampling plays a role for evaluations in the elimination context. There was general consensus that the details of the random protocol could be developed as we move forward with mapping in the higher risk areas, where the 1st-line village approach is most likely to work.

*Additional detailed discussion on mapping strategy.*

The group agreed that it is worthwhile to invest in rigorous mapping up-front, to avoid unnecessary costs later. It was further agreed that:

1. If the location of breeding sites is unknown, random sampling of villages may be used.
2. If purposive sampling of 1st-line villages fails to identify transmission, random sampling of the remaining evaluation area should be performed
3. In order to minimize the chance that areas in need of MDA are falsely classified by the mapping strategy as 'non-endemic', it was determined that a conservative sampling approach that may result in over treatment of some areas is preferable. Therefore, sampling strategies and thresholds that lead to over-treatment are preferable to those which might lead to under-treatment. If data become available that suggest that certain areas did not need to start MDA because the threshold used was too conservative, programmes can stop MDA without additional evaluation.
4. Local knowledge will continue to play a role in selecting or excluding areas for mapping. The reasons for exclusion should be clearly documented for inclusion in the verification dossier.
5. The choice of the mapping unit (district or sub-district) is a decision the country will need to make based on what is known about transmission potential in the surrounding areas. Sub-district approaches will be important in populous areas so as to avoid having to treat large populations that may not be at risk for infection.
6. Both males and females should be tested.

<u>iv. Discussion: Choice of diagnostic test</u>

The group agreed on the use of Ov-16 RDT as primary diagnostic at the moment. Requiring the use of Ov-16 ELISA would be resource intensive. As the concern with the RDT is sensitivity, any mapping area that exceeds the threshold for starting MDA should start MDA. Ov-16 ELISA would be indicated as a secondary diagnostic, for verification in areas where Ov-16 RDT positive results are below the threshold for starting MDA. Skin snips lack the required sensitivity and people are more reluctant to undergo snipping in areas where symptomatic disease is not common, so snips would not be a component of the standardize protocol.

*OTS Recommendations: Choice of diagnostic test*

1. Primary test is the Ov-16 RDT, though countries may opt for ELISA only

2. Dried blood spots should be collected so that if RDT results suggest that treatment is not indicated, then the more sensitive Ov-16 ELISA can be used to confirm.

v. Discussion: Threshold for starting treatment

The threshold for starting treatment has to be divided into two separate discussions. In areas with purposive sampling, it would not be appropriate to combine results across villages, so there would need to be a community-level criterion. It was thought that a minimum of three 1st-line villages should be evaluated with 100 adults per village. Up to 5 or more villages could be included. For areas where random sampling is used, the results could be combined across villages and thus evaluation mapping area level threshold would be needed. To create a village level threshold for the random sampling area could require very large sample sizes. As the point of mapping is to efficiently and effectively determine where MDA needs to be implemented, this was not felt to be a reasonable option. Additionally, as transmission may be focal, it would make more sense to sample more villages to increase the chances that a signal is detected. However, this must be balance with the need to keep sample sizes at a reasonable size, which would require smaller numbers of people recruited in each village as larger number of villages are selected. It was unclear what number of villages should be selected, but 30 village cluster surveys are common so that could be a tentative starting point. The appropriate number of clusters and sample size for the systematic random sampling will depend on the threshold being tested, the performance of the diagnostic test used, and the desired precision and power of the survey. Consideration should also be given to the size of the evaluation area, cost and the focality of the disease. Modeling and simulations based on available mapping data can help to inform these decisions moving forward.

Models suggest that the population prevalence of infection required to sustain transmission of onchocerciasis is highly dependent on the annual biting rate, which is typically highest in the areas of higher prevalence. Thus, the minimal prevalence needed to start MDA in low transmission areas is expected to be higher than the prevalence required in hyper- and meso-endemic areas. Models of transmission in low prevalence areas should be interpreted with caution as there is not much empirical evidence from these areas to support or validate the models. One of the more conservative estimates was 5%. The OTS felt that it should be more conservative, particularly if a less sensitive test such as the Ov-16 RDT is to be the primary tool. Consensus was that a tentative threshold would be 2-2.5%, with the expectation that the upper bound of the one-sided 95% confidence interval would need to be less that the threshold. It is important to note that this threshold needs to be validated, but it was felt to be conservative enough to allow mapping to proceed and sample sizes to be determined that would be larger than what would be needed if a higher threshold is determined to be appropriate. The fastest way to begin to validate the threshold to start MDA would be to collect flies in untreated areas with a range of seroprevalences. If infective flies are never found below threshold ‘A’ but are always found above threshold ‘B’, the appropriate threshold would lie somewhere between the two. The data collected could be fed into models of transmission for further refinement of the threshold for starting MDA.

*OTS recommendations: Thresholds for starting MDA*

1. Use a provisional threshold of 2% for starting MDA
2. Use village level thresholds for starting MDA when purposive selection of villages is used
3. Use district level thresholds for starting MDA when random selection of villages is used but support operational research to compare district level to village level approaches should be considered

4. Random sampling within villages should be used for the district-level approaches
5. Prioritize validation or refinement of the 2% threshold with an urgent need to begin to perform entomological studies to confirm the presence of transmission at different levels of seroprevalence
6. More operational research is needed to finalize the number of clusters and cluster size for the random approach and to determine if village level or evaluation area level thresholds should be used; this approach is ready for operational research but there was not consensus on an approach for programmatic use
7. The maximum evaluation area size needs to be determined for both elimination mapping and stop-MDA surveys; this may be based on a geographic area or population size; geographic area may make the most sense given the known average flight range of the vector species

vi. Other discussion

The need to develop criteria for the inclusion or exclusion of urban areas in evaluations was brought up. Although in general, urban areas are not thought to provide suitable habitats for the vector of onchocerciasis, there are some urban areas in central Africa that are known to have transmission. Programmes need to consider this issue careful and it will need to be addressed at a future meeting of the OTS. A second issue that will require discussion at a future meeting of the OTS is whether mapping protocols for LF and onchocerciasis can be harmonized. This will require the use of a similar threshold and target population, so this discussion was postponed until addition details on the sampling strategy are finalized.

## 6. General Outline of the Elimination Mapping Protocol

1. Determine areas that may be excluded from mapping.
2. Identify areas where transmission is most likely for the initial elimination mapping and then move out to areas where transmission is less likely
3. Determine the evaluation unit (district or sub-district), this may vary depending on the context of the evaluation
4. Begin with evaluating 3-5 purposively selected 1st-line villages and a minimum of 300 people
    - Use the Ov-16 RDT (countries may opt to use ELISA; either the monoplex or biplex RDT is acceptable)
    - Sample adults ≥ 20 years old
    - If the seroprevalence in a village exceeds 2% then initiate MDA in the evaluation unit
    - If the RDT results are less than the 2% threshold, then they should be confirmed by ELISA
5. If purposive sampling cannot be done or if transmission is not identified by purposive sampling, a random sampling evaluation should be performed
    - Sample adults ≥ 20 years old
    - Use the Ov-16 RDT (countries may opt to use ELISA; either the monoplex or biplex RDT is acceptable)
    - Consensus on the protocol for this was not reached
    - For research purposes, protocols should be designed that enroll people from 30 clusters with an appropriate cluster size to detect an evaluation unit level seroprevalence of 2%
    - If the upper bound of the 95% confidence interval of the random sample excludes the tentative threshold of 2%, then MDA is not needed
6. If the threshold for starting MDA is raised based on data collected during operation research of the initial pilot, programmes may stop MDA in evaluation units that would not have exceeded the new threshold.

## 7. Routine Monitoring and Evaluation (M&E)

Presentation: M&E

Routine M&E should be quick, inexpensive, and useful for informing programmatic decisions. It may be possible to design routine M&E in such a way that it could also be used as a pre-stop-MDA survey. Evaluation areas that passed such a pre-stop-MDA evaluation would then proceed with a stop-MDA urvey. Those that failed would still obtain actionable M&E information and meet the requirement specified in the 2016 WHO guidelines that requires M&E at least every 4-5 years.

i.Ethiopia protocol for M&E

Ethiopia conducts M&E in villages that have conducted at least 10 years of MDA. Using baseline REMO data, the programme selects 2-3 communities with the highest nodules rates. If baseline data are not available the programme visits communities with 10km of the river for assessment. Skin snip biopsies are taken from adults ≥ 20 years old and are evaluated by microscopy and DBS are collected from children 5-10 years old. If >2% of adults or 1% of children have positive results, transmission is considered to be ongoing and the treatment strategy is adjusted. Ethiopia also performs more in-depth impact surveys, which are stop-MDA surveys. The evaluation is performed in a transmission zone that may encompass more than one district, as long as the treatment history is similar. Communities are purposively selected along rivers and the evaluation continues away from the river until the sample size is reached. This can be combined with entomological surveys in order to make the assessment a full stop-MDA-survey.

ii. Pre-stop-MDA survey

One approach that was proposed was to survey three sentinel 1st line villages in a district (or other evaluation unit). Sampling would be purposive, so that programmes could select villages at the highest risk for ongoing transmission, could take geographic distribution into account if they felt that is was important, and could include villages of concern as well. At least 300 children ages 5-9 years would be included. A convenience sample of 100 children per community would be selected. If a village had less than 100 eligible children additional villages would be added until at least 300 children were evaluated. Either RDT or ELISA could be used for the testing. The threshold for proceeding to a stop-MDA survey will have to be determined, but this can be tested moving forward by tracking the number of areas that pass the pre-stop-MDA survey that also pass the stop-MDA evaluation.

iii. Entomology

The 2016 guidelines require entomologic M&E but do not fully describe what is expected. Although routine capture and analysis by PCR would be informative, many programmes do not currently have the capacity for the PCR analysis. None-the-less, much important information could be obtained as part of entomological M&E without PCT analysis. For stop-MDA evaluations for PTS, programmes will need to identify breeding sites and determine the seasonality of transmission. It would also be helpful to determine parity rates. Determination of biting rates would give some indication of potential intensity of transmission even in the absence of knowledge about the infectivity of the flies (e.g. areas with high biting rates would be expect to require more intense interventions to interrupt

transmission). Associating 1st line villages and other villages with particular breeding sites is important and countries should create line lists of these associations.

iv. Presentation: Coverage surveys and tools.

As achieving high coverage is required to successfully interrupt transmission of onchocerciasis, accurate estimates of coverage are important. A coverage survey requires no laboratory capacity and can be used to improve programme impact because it allows programmes to detect and respond to deficiencies in MDA coverage. Standard protocols for coverage evaluation surveys and rapid assessment have been recently developed and endorsed by the WHO Strategic Technical Advisory Group and WHO is expected to publish guidance in the near future (although some tools are available on the NTD Support Center website). The surveys are designed to assess an administrative area (e.g. district) using probability proportionate to size methodology to select villages for evaluation. Within villages, households are selected by random selection of a defined segment of the villages, and all households within the segment are enrolled. Resources are listed below.

Coverage survey builder:
http://www.ntdsupport.org/resources/coverage-survey-builder-coverage-evaluations.
Supervisor's coverage tool:
http://www.ntdsupport.org/resources/supervisors-coverage-tool
Coverage survey analysis tool:
http://www.coverage.linkssystem.org
Data quality assessment:
https://www.ntdenvision.org/resource/publication/data_quality_assessment_dqa_for_ntds

The data can be uploaded into the integrated NTD database.

*1. Presentation: Nigerian Experience with Coverage Tools*. The Nigerian programme was trained in-country on the use of some of the WHO-supported tools and then the NTD Steering Committee adopted the protocols and they were adjusted for local conditions.

The Supervisor's Coverage Tool is a quick and inexpensive tool that classifies coverage as being likely above or below a threshold. This allows for rapid identification of problem areas, which can be targeted for mop-up activities. It was implemented in 3 states immediately following MDA. A team could evaluate one supervision area in 1-2 days. The evaluation found that coverage was classified as 'inadequate' or 'cannot conclude coverage was good' in all areas. It was determined that many schools that were targeted for MDA did not implement MDA, there were unregistered schools that were not included in MDA planning, and there was a high rate of absenteeism in places where coverage was poor. Additionally, some residents in one STH-endemic local government area attended school in a non-endemic local government area and thus were not treated.

The more in-depth Coverage Evaluation Survey was implemented in a subset of local government areas (LGAs) within the Nigerian states. The number of LGAs evaluated depended on the number of such areas within the state. Evaluations occurred in 10 LGAs in 4 states in 2016, with surveys in 9 states planned for 2017. For ivermectin, the survey coverage was less than reported coverage in 8 LGAs. For albendazole, the survey coverage was less than reported coverage in 7 LGAs. For

praziquantal/ mebendazole, the survey coverage was less than reported in 3 LGAs. For ivermectin and albendazole, survey coverage was less than the goal coverage in 50% of LGAs. For praziquantel, survey coverage was less than goal coverage in 78% of LGAs. Investigations behind the low coverage rates revealed a variety of factors. In some areas entire communities were not treated because drug distributors were not identified and communities were not mobilized. In one area, inappropriate drug distributors had been selected that were not trusted by the community. There were also complaints about the lack of financial incentives for drug distributors. Finally, the survey identified issues with identifying the correct population denominator, with an underestimate of the population for the reported numbers compared to the survey numbers.

v. Discussion: Routine M&E

The advantage of the pre-stop-MDA survey is that it could serve two purposes: provide actionable data throughout the life of the programme and help programmes decide when it might be worth the investment to perform the stop-MDA survey. As this approach has not be used before, it would be necessary to set targets arbitrarily or based on data collected for other studies and adjust the strategy moving forward. It should be noted that this approach uses purposive sampling and is not designed to determine an overall prevalence of infection but is designed to obtain a quick look at transmission in high risk communities. The strategy employs a community-based strategy to align with the known epidemiology of onchocerciasis and was felt to be a more representative assessment of transmission. The sampling is convenience sampling, in contrast to the random sampling required by the guidelines for a stop-MDA-survey. The 5-9 year-old age group was chosen, because it was felt that including younger children would dilute the signal because they are at lower risk for infection than the older children. As only serology is used, there is no need to time the survey around MDA because the use of ivermectin will not impact antibody results. If the pre-stop-MDA-survey were to be used as routine M&E, then programmes may consider performing a survey every 3 years and perhaps rotating the villages evaluated if they need to cover a large geographic area. There are a variety of operational research questions that would need to be evaluated moving forward; those questions are listed below in the OTS recommendations section.

It was pointed out the performance of coverage surveys and the supervisor's coverage tool in Nigeria allowed identification both problems with the coverage data as well as potential solutions. As it is known that programme impact is dependent on coverage achieved, the underperforming areas should focus on improving coverage. An impact survey involving serology would not be needed until after the coverage is improved. Additionally, there would be no need to consider changing to a strategy of MDA twice a year until after coverage is improved to goal. Countries will need to determine how to balance the cost of coverage surveys in districts currently under MDA with the need to expand MDA into untreated areas.

OTS recommendations: Routine M&E

1. Programmes should continue to make use of opportunities to collect M&E data even in the absence of a defined strategy. Many countries have integrated assessment with other diseases assessment (e.g. lymphatic filariasis or soil-transmitted helminthiasis) and received information about the status of transmission that allowed the programmes to prioritize activities.

2. WHO should consider how to define the geographic area that can be included in a single stop-MDA evaluation. One potential way to break areas down into manageable units would be to use knowledge or river basins and breeding sites and the average flight range of the vector to define an evaluation area. Input from entomologists will be needed.
3. Operational research to compare school-based assessment to community-based assessments is needed. In some countries schools draw from a single village; in others schools draw from multiple villages, so school-based assessments may not be appropriate in all settings.
4. WHO should pursue the development of a pre-stop-MDA-survey for onchocerciasis based on 1st line villages as described above.
5. Operational research is needed to predict which threshold best predicts whether an area that passes a pre-stop-MDA-survey will also pass a stop-MDA-survey.
6. Coverage surveys should be considered as an important M&E tool that can be used even in the absence of laboratory capacity to make programmatic decisions.
7. Coverage needs to be verified before considering a change in the frequency of ivermectin dosing because experience has demonstrated that poor performance is often due to inaccurate reported coverage.

**8. Stop MDA Surveys**

i. Presentation: Modelling of serological thresholds for stopping MDA

Thresholds for Ov-16 ELISA serology to assess whether MDA can be stopped were modeled using ONCHOSIM, an individual-based model which predicts expected trends in infection indicators in a single community. The model includes measurable variables and imputed variables and allows for chance events. The ONCHOSIM model presumes a closed system in which there is no movement in or out of the area.

Assumptions for the model were as follows: i) anti-Ov-16 seroconversion is immediately triggered by the appearance of the first mature worm , ii) Ov-16 tests vary in both sensitivity and specificity, iii) sero-reversion either does not occur, or it occurs immediately, iv) 5% systematic non-compliance with ivermectin treatment.

Key findings based on 750 simulated scenarios were as follows:

i) the most informative age group is 5-14 years

ii) the younger than 5 years old age group is not informative and should be excluded from the sample;

iii) the predictive value of the model depends on the probability of elimination, which means MDA frequency and coverage or a pre-stop-MDA survey are important;

iv) the threshold indicative of interruption of transmission depends on baseline endemicity; the critical threshold declines with higher baseline endemicity, which is indicative of more intense transmission

v) the assessment threshold should be a conservative one and the highest-risk locations (first-line villages) should be assessed first;

vi) the estimated adjusted threshold for the 5-14 year-old age group is around 1.2% with 60% sensitivity and 100% specificity for identifying interruption of transmission; this threshold assumes rapid sero-reversion after elimination of infection.

The reliability of these predictions was limited by uncertainties about the Ov-16 dynamics (e.g. the trigger of antibody production, time to sero-reversion) and sensitivity and specificity of the test. Also, simulations were done for a limited number of settings. It was also noted that ONCHOSIM's predictions in general are more optimistic about elimination prospects than those of EPIONCHO. There was a large range of potential thresholds depending on baseline endemicity and the assumption about whether or not sero-reversion is seen in children. Under the no seroreversion assumption, the unadjusted threshold was a high as 6.7% in areas with baseline CMFL ≥ 80. Under the seroreversion assumption, the threshold was 1.9% in these areas. Adjustments for potential misclassification result in a threshold of 1.2%.

Discussion: It was debated about whether changing the age group sampled from the 5-9-year-old age group to a 5-14-year-old age group would create logistical difficulties. It would certainly make it hard to integrate evaluations with other disease evaluation and the proportion of children 5-9 and 10-14 would have to be carefully balanced. Higher serological thresholds compatible with interruption of transmission were presented. If sero-conversion were triggered by mf production, the threshold would be lower and its predictive power would be less. If the assumption is that sero-

reversion is slow or does not occur, the threshold is much higher. Concerns that some of the potential thresholds were quite high in the lower transmission areas were expressed. There was general agreement that it would be best to be conservative, particularly as ONCHOSIM is considered more optimistic than other models, and chose a threshold that was low and that would be valid in most circumstances. It may be possible to develop different threshold for different intensities of transmission, but empiric data to validate an increase from 0.1% to 1% would be needed prior to additional changes. Uncertainty about coverage and compliance implies that impact on infection always must be evaluated through epidemiological surveys. Some felt that adding entomology results to the model would provide additional information for decision making on when to stop MDA. As there is concern about increasing the age group to include older children (the older the children the higher the acceptable threshold), a more detailed comparison of the 5-9 and 5-14 year-old age groups would be helpful.

OTS Recommendations: Serological Threshold

- Studies that evaluate 1% as the serological threshold for stopping MDA should be encouraged. Validation of the threshold would require that study areas pass entomological criteria at the stop-MDA point and after three years of PTS. Annual evaluation of serological and entomological indicators would be important in this research setting.
- Comparing seroprevalence and logistics of recruiting different age groups would be helpful.
- Entomologic studies could be used to help evaluate any new threshold

ii. Presentation: Adjusting Sample Size for Test Performance

WHO's 2016 Guidelines indicate that the sample size for the epidemiological component of the stop-MDA survey should be sufficient to measure <0.1% Ov-16-seropositivity with 95% confidence. This guidance assumes the use of Ov-16 ELISA rather than RDT and a perfect test. It also does not take into consideration that power to detect the desired outcome. These assumptions should be examined more closely. Based on published and unpublished data, we know that the Ov-16 ELISA has a sensitivity of 50-90%, with 80% appearing to be a reasonable estimate for ELISA. There are a variety of published specificities and sensitivities, ranging from 96-99.7% depending on the test and format. The performance characteristics of the ELISA used by OEPA have not been published. A test with 99% specificity cannot measure 0.1%. In fact, a test with 99.98% specificity has only a 55% chance of finding 0 positive results in 3000 uninfected people. It would be nice to have a system that would allow for some false positive results without requiring additional testing. The power of the current strategy to pass an evaluation when transmission has been interrupted is only 22%. This means that the majority of sites would fail an evaluation when they should have passed.

If we wish to maintain the current threshold in which the UL of the 95% CI is <0.1%, and we assume 80% sensitivity and 100% specificity of the Ov16 ELISA, then the required sample size would be 3,660 if no positives were allowed. Samples sizes of 11,075 and 17,320 would allow 4 and 8 positive results and would have power of 54% and 75%, respectively. If a cluster sample design is used, further increases in the sample size in order to compensate for design effect—a measure of correlation of data within the cluster—would be required. Adjustment for design effect would not be needed if no positive tests are allowed.

Discussion:

The discussion about thresholds continued into the discussion about sample sizes. If the threshold were to be increased, it would be possible to allow the development of critical cut-offs (e.g. allow a few positive tests to account for imperfect specificity). Many thought standard critical cut-offs should not be applied uniformly across all countries and contexts, much like seroprevalence thresholds. NOECs would need to be able to adopt (e.g. lower) the cut-offs for their specific contexts when justifiable. However, at the moment, it was agreed that a single threshold should be the starting point and that WHO would need to create a framework for adapting thresholds when justifiable. Although the presentation focused on a district or sub-district level prevalence, it was pointed out that it may be important to develop a community-specific cut-point, particularly for the high prevalence 1st line villages. An increase in the threshold would reduce the sample size required (see previous discussion on next steps for increasing the threshold).

Finally, there was a discussion of how sample size depends on many things: threshold, sampling methods, age group sampled, and performance of the test being used. As there are potential changes to all of these variables, it was not possible to determine what the appropriate sample size for the current methodology should be. However, there was clear consensus about 2000 children being too few to measure 0.1% seroprevalence. As 3000 children had been used in settings in where programmes then stopped MDA and successfully completed 3 years of PTS without evidence of recrudescence, in the absence of new information, it was recommended that WHO require a minimum of 3000 children (unless there are fewer than 3000 children in the survey area).

OTS Recommendations: Sample size

- OR is needed to validate the choice of design effect for different sample sizes and critical cut-offs if a cluster design is to be used
- Programmes should sample at least 3000 children when performing a stop MDA survey, unless fewer than 3000 children live in the survey area.

iii. Presentation: A comparison of purposively selected first-line villages vs. randomly selected villages.

Surveys integrating transmission assessment surveys for LF with M&E for onchocerciasis (iTAS) were conducted in Nigeria and Burkina Faso, in sites which had received ≥12 years of treatment with IVM and had met criteria for LF TAS. First a pre-iTAS assessment took place, in which a LF sentinel sites were evaluated along with onchocerciasis 1st-line villages that served as spot check sites for the LF evaluation. In those areas that passed the pre-iTAS for LF, a random cluster survey was conducted with random sampling among children ages 5-9 years. The sample size for the iTAS was larger than for routine LF TAS, because of the addition of children outside the 6-7 year-old agree group used for LF TAS. Three diagnostics were used with all participants: the Ov-16-Wb-123 Biplex RDT, the filariasis test strip antigen RDT (FTS), and DBS (for future testing with laboratory-based ELISA).

In Nigeria, both surveys were conducted in four districts. The pre-iTAS was conducted in four purposively-selected villages (3 onchocerciasis 1st line villages and 1 LF sentinel site) per district, with children ages 5-9 in two of the three onchocerciasis 1st-line villages and people ages ≥5 years in the LF sentinel sites and the 3rd onchocerciasis 1st-line village. Around 300 participants were sampled in

the LF sentinel site and 3rd onchocerciasis 1st-line village; 100 children ages 5-9 were sampled in the other two 1st-line villages. If the pre-iTAS was passed, the iTAS was conducted in 30 primary schools selected systematically from the entire district, with children ages 5-9 years, with a total of 3,000 children per district.

In Burkina Faso, both surveys were conducted in three districts using the same design except that only 100 people per village were evaluated in the pre-iTAS and age groups varied by district not by village type.

It was found that onchocerciasis prevalence (as measured with the Ov-16 RDT) among the same age group was similar across purposively-selected villages and randomly-selected sites (schools or communities). Some LF sentinel site villages had higher onchocerciasis prevalence than the onchocerciasis 1st-line villages among children in the 5-9 years old. In Burkina Faso, where adults and children were evaluated for onchocerciass during the pre-iTAS, some older age groups in non-1st-line villages had substantially higher Ov-16 seroprevalence than the same groups in 1st-line villages. As expected the proportion of Ov-16 RDT positives increased with age; using a sampling strategy that includes older age groups will result in a more conservative pre-stop assessment. In no case was the 1st-line village seroprevalence higher than the district-wide prevalence, suggesting that a purposive pre-stop MDA survey approach might be reasonable, as one would expect districts to fail a stop-MDA survey if the 1st-line villages cannot meet the criteria. These results should be interpreted with caution as the analysis is preliminary and ELISA results are pending.

Discussion: There was a lot of discussion about the results in 1st-line and non-1st-line onchocerciasis villages. It is possible that some of the LF sentinel villages were also 1st-line villages for onchocerciasis, though the country programmes confirm that they were not. The findings could be the result of misidentified 1st-villages due to changes in the locations of breeding sites or perhaps lower coverage in non-1st-villages. In either case, it seems clear that transmission is not completely understood in the areas surveyed and that both the 1st-line village approach and the random cluster approach may play important roles in the evaluation of transmission. Whether 1st-line villages should be evaluated in a pre-stop-MDA survey followed by a random evaluation of the remaining villages in the evaluation area or followed by a more in-depth survey of 1st-line villages in combination with a random survey of non-1st line villages remains unclear.

OTS Recommendations:

1. More details about the comparison of seroprevalence of Ov-16 in 1st-line and non-1st-line villages should be provided to the OTS. If the findings remain unchanged, particularly after Ov-16 ELISA testing is completed, serious consideration for a two-stage approached to the evaluation of onchocerciasis transmission would be indicated.
2. A pre-stop-MDA evaluation of 1st-line villages should be developed.

Iv Presentation: Uganda's Experience with Stop-MDA Surveys

Uganda has interrupted transmission in 15 out of its 17 foci. The onchocerciasis elimination programme combines vector control using the larvicide Abate and Mectizan distribution. The country has two distinct vectors. One is *Simuliun neavei*, which is the main vector in most of the foci. It has a shorter flight range than *S. damnosum*, does not fly into open areas, and breeds in small to

medium-sized streams only, making it easy to target with larvicides. Its larvae attach exclusively to fresh-water crabs. As some of these crabs have disappeared, possibly due to deforestation, the vector has disappeared in some areas in the absence of vector control. The other vector is *S. damnosum* sensu latu. It is present in the northwest of the country, has a longer flight range, and is more difficult to control using larvicides.

The Uganda onchocerciasis programme receives input on stopping MDA from its Uganda Onchocerciasis Elimination Expert Advisory Committee (UOEEAC). Recommendations are voted upon by committee members. Although only a simple majority is required, the decisions are usually by consensus. Because Uganda engages in vector control and elimination, guidelines for entomology evaluation after vector elimination were needed. The MOH's national onchocerciasis elimination guidelines (2011) provide guidance on how to demonstrate the absence of vector (as determined through examination of flies and/or crabs) in *S. neavei* foci.

Serological evaluations are performed using DBS and OV-16 ELISA. DBS are collected from children selected by a multistage stratified sampling scheme at the parish level (several administrative units below the district level). Sampling is challenged by difficulties in conducting a complete census of the survey population, which means that people will always be missed and by difficulties in sampling when there are fewer than 3,000 children present. As Ov-16 ELISA cannot differentiate between old and new infections and infection and exposure, a method for evaluating small numbers of positive Ov-16 ELISA results, which could represent exposure or infections that could not contribute in the future to the transmission of onchocerciasis, is used. When there are fewer than 10 children with positive Ov-16 results, PCR of skins snips is performed. Challenges in implementing this include the need to find the children which is difficult and time-consuming if not done immediately, parents' resistance to allowing their children to be snipped. It is important to collect parental and location information during the survey to facilitate finding the children at a later date.

Entomological evaluations involved blackfly collection and evaluation of the heads of the collected flies using poolscreen O-150 PCR. Flies are collected by conventional human landing catches carried out twice weekly. Challenges include removing non-vector species from the pool of collected field, issues with substandard preservation of fly samples, and storing too many flies in one container which can lead to breaking up of parts needed for identification. Proper identification of flies prior to laboratory analysis is essential.

Given the absence of (*S. neavei*) flies in the country's southern and eastern foci, the laboratory component of stop-MDA surveys conducted thus far have focused on the epidemiological component (Ov-16 ELISA), with skin snip PCR for any Ov-16 positives. PCR of blackflies has mainly been used to identify the species of the flies caught. Fly dissection is used only to assess the parity rate.

Discussion:

The committee asked for more details on the sampling frame used for serologic evaluations. The primary sampling unit is the parish (which is a collection of villages smaller than a district). When foci are small, all parishes are sampled. When foci are large, a simple random sample of parishes is

obtained. A census of the selected parishes is made and a simple random sample of eligible children is evaluated using Ov-16 ELISA. The OEPA protocol is used for the ELISA, which recent studies have suggested exhibits a sensitivity of roughly 43% and a specificity of 99.98% under operational conditions. If fewer than 10 children are positive, they are evaluated by collecting skin snips and performing skin snip PCR; all children tested have had negative PCR results. Concerns were expressed about the validity of skin snip PCR as a confirmatory test given that it is less sensitive than serology (even though it could detect patent infections), the children must be located, and ivermectin use influences the results. The consensus was that it would be beneficial to replace it with another type of test (e.g. an independent antibody test or an antigen test) and that it is important to (re)test children at least 11 months after their last dose of ivermectin.

Concerns were expressed about the relatively few positive results by Ov-16 ELISA. Experiences from other countries have revealed issues with the quality of dried blood spots. Poor handling of the DBS could result in false negative results. To address this concern, Uganda has performed some QA/QC through the assistance of the University of South Florida. Both positive and negative controls are used on a subset of ELISA runs. The data are received and remotely reviewed by Dr. Thomas R. Unnasch. As yet, however, no standardized QC system is in place. It was felt that a proficiency test or standardized QC would be beneficial. It was noted that the Uganda programmes has had many positive DBS from areas of ongoing transmission in the Democratic Republic of the Congo.

The speaker was asked how the programme ensures that the vector collection is implemented in a representative manner. It was discussed that this can be difficult to do, though catching points are selected by taking into consideration the whole focus or river basin. It is unclear what criteria should be used to select one catching point over another and how many catching points should be evaluated in an areas. It was suggested that it might be best to define a geographic area based on the average flight range of the black fly to help determine the number of sites that need to be sampled. In Uganda, because the larvae of *S. neavei* are carried on freshwater crabs, the programme has had to develop a system of crab surveillance. In some areas the crabs have disappeared. Their disappearance is presumed to be due to a pathogen in the rivers, rather than to environmental causes; the crabs are starting to return to some streams.

The speaker was asked about how the programme manages refugees and cross-border foci. Many South Sudanese refugees have settled into Ugandan communities rather than stay in refugee camps; the UOEEAC has recommended that the refugees be screened with Ov-16, and treated if need be, but it is unclear whether there has been follow-up on these recommendations. The Uganda Ministry of Health in consultation with the Office of the Prime Minister has developed a Comprehensive Refugee Response Framework that will guide all activities in the camps and nearby communities. UOEEAC recommendations will be implemented under this framework. Certain foci will not be able to reach elimination targets until control is achieved on both sides of the international border; Uganda has been making progress in collaborating with DRC and South Sudan. Recently Uganda has co-implemented both serological and entomological evaluations in cross-border foci shared with DRC. The results obtained are helping both programmes to understand the transmission dynamics in these foci.

<u>OTS Recommendations</u>

- An alternative to skin snip PCR for testing of Ov-16 ELISA positive survey participants needs to be developed (e.g. antibody test or antigen test)

- WHO should develop a standardized system of QA/QC for ELISA once a single ELISA is selected for support

v. Presentation: Other Stop-MDA Protocols

This presentation reviewed stop-MDA survey protocols from five countries—Guatemala, Sudan, Togo, Burkina Faso, and Ethiopia—noting lessons learned from each. The presentation also discussed a newly proposed protocol.

For each protocol, the selection of localities (primary sampling unit [PSU]) and the selection of participants (secondary sampling unit [SSU]) were examined. For the PSU, the assessment area (district, multiple districts, state, transmission zone, etc.), primary sampling unit or locality (first-line villages, all villages, schools, potentially endemic communities, etc.), sample size (number of localities per assessment area to be visited), and selection method (purposive sampling, simple random sampling, systematic sampling, stratified sampling, PPES) were noted. For the SSU, the participants (children, adults, certain age ranges), sample size (number of persons per locality), selection method (census, convenience sample, simple random sampling, systematic sampling), and diagnostic test (ELISA, RDT, skin snips) and the related threshold were noted. The summary here focuses on the Ov-16 (ELISA or RDT) epidemiological component of these surveys, though it was noted that in several cases other tests (e.g., PCR screening of blackflies, clinical eye examinations, skin snip microscopy, nodule palpation, and/or skin examination) were performed.

1. The Guatemala protocol presented was used in three of the country's four foci, each of which was made up of several *municipios* (equivalent to counties). The PSU was schools in potentially endemic communities within each focus; the potentially endemic communities each had at least one of the following characteristics identified from historical data: past evidence of onchocerciasis transmission, suspicion of past transmission, or current twice-yearly IVM MDA. Schools were selected until the sample size of students was reached or exceeded, using simple random sampling from among all schools in the focus. The SSU was schoolchildren 6-12 years old, with 3,000 children per focus plus an additional 30% to account for non-response. DBS were taken for Ov-16 ELISA testing. The advantages school-based recruitment is that is allows for more rapid recruitment of the needed number of children. The disadvantage was that expansion of the PSU to reach the target SSU sample size in one focus may have resulted in inclusion of some participants from non-endemic communities. All 3 areas passed the evaluation, with no children with positive OV-16 results out of more than 12,000 children tested.

2. The Sudan protocol was used in a single focus, with schools in and around the focus selected as the PSU. These included participants from communities within and close to the focus, as well as from communities of displaced people within and around the focus. (A dam had been built, displacing some of the population in the transmission area.) The SSU was schoolchildren ages 5 or 6 to 10 years old, and the target sample size was >3,000. DBS were taken for Ov-16 ELISA testing. There were no children, out of more than 6,756 tested, who had a positive Ov-16 ELISA. There were also no positive pools from the 17,537 flies that were tested by O-150 PCR.

3. The Togo protocol was used in four endemic districts, with a PSU of at least 10 villages per district. The selection method within each district was stratification into first-line villages and other villages,

using PPES and giving priority to first-line villages. If 10 villages were not recruited in a district, the remaining villages were recruited from another, preferably adjacent district. The SSU was children 2-9 years old who were "native" to selected villages, with a final sample size of 3,000-4,000 to ensure recruitment of 2,500 to 3,000 children after refusals and absences. Children were recruited in the community in a random sample; if the required number of children exceeded the total population of the village, then a census of all children 2-9 years old in the village was recruited. DBS were collected for Ov-16 ELISA testing. Results were not available at the time of the meeting.

4. The Burkina Faso protocol was used in two contiguous districts where recrudescence had occurred. The evaluation area considered all inhabited villages and hamlets within 10 km of both sides of the river within this endemic area. The PSU was the village, with 40 villages selected purposively. The SSU was children <10 years old, with a sample size of 3000 for testing with Ov-16 RDT. An additional sample of 250-300 individuals ages ≥5 years, per village, was targeted for testing with skin snip. The number of children sampled for Ov-16 RDT in each village was proportional to the number of children in each village compared to the total number of children in the full 40 villages; the children were to be sampled randomly. For skin snips, all members of selected households were evaluated until the sample size was met. Any children testing positive with Ov-16 RDT had skin snips taken for PCR testing. Results were not available at the time of the meeting.

5. The Ethiopia protocol is proposed for use in districts that are part of a given transmission zone. The assessment area is the district. Contiguous districts with similarly baseline endemicity and history of ivermectin treatment may be combined into one evaluation if they are in the same transmission zone. The PSU is all communities within the district(s) chosen. The number of resident children 5 to <10 years old in each village is estimated to generate a district-level estimate. The number of communities to visit for the evaluation is determined by adjusting the target of 3,000 children by the expected enrollment rate. If the boundaries of assessment area are clear, then communities are selected randomly from all communities in the area; if the boundaries of the assessment area are not clear, then communities are purposively selected along rivers and then moving outward until sample size is achieved. The SSU is children ages 5-9 years old. A minimum of 2,000 children are evaluated, though the goal is usually 3,000 children. All children who meet the age criteria within each selected community are tested. The test is the Ov-16 ELISA. The stopping threshold is the one specified in the 2016 WHO criteria.

6. Proposed risk-stratified stop-MDA survey . One final protocol was presented. It is a proposed risk-stratified stop-MDA survey. The assessment area varies depending on the country context. Districts can be merged to form assessment areas, provided that districts are contiguous, share a river system, and take into account other contextual factors as advised by national onchocerciasis expert committees (NOECs); if districts are combined, the survey results would apply to the assessment area as a whole, not for the individual districts. The PSU is the village, which would be categorized into high risk and other villages. High risk villages would include 1st-line, and villages of programmatic concern. Villages of programmatic concern could include those with historical evidence high prevalence, those with poor MDA coverage, those that are in close proximity to a border area, and those villages that should be included in order to ensure geographic distribution across the assessment area. Once all villages are identified, the number of resident children 5-9 years old in each village is estimated and the assessment total is calculated. The anticipated sample

size is adjusted for the expected rate of refusal. At least 30 villages should be included in the evaluation, though if more villages are to be assessed, the adjusted sample size is divided by the number of villages to determine how many children should be sampled in each village. Villages are sampled systematically from a list of all villages in the assessment area according to perceived transmission risk (ordered from highest to lowest), taking a random starting point and using a sampling interval to select villages from the list.

The SSU is children ages 5-9 years. A minimum of 3,000 children would be evaluated per assessment unit; this number should be adjusted for sensitivity of the Ov-16 serology test and the expected non-response rate. For example, if the sensitivity of the ELISA was 80%, a sample size of 3680 would be required, depending on population size. An expect non-response rate of 5% would increase the sample size to 3864. This sampling scheme would still require that no children have a positive serological test. If the sensitivity was 60%, the sample sizes increase to 3890 to 4960, not including the adjustment for non-response. Neither of these adjustments improves the power of the survey, which is around 23% (as compared to the LF evaluation, which has a power of 75%). Low power means that an evaluation area where the threshold has been met may still fail the evaluation. A power of 23% means that 77% of evaluation areas that should meet the stop-MDA criteria will fail to do so (which means that MDA will continue when it should actually be able to stop). Children are selected by systematic sampling, with a fixed proportion of children to be recruited using a predefined sampling interval coupled with a walk through using a set route through the village to select households and participants.

The official guidance from the 2016 guidelines on sampling is unclear. The document states that there should be a 'multistage stratified sampling method scheme applied to the local lower administrative unit level'. However, review of the literature on the stopping surveys only found this methodology mentioned once in a paper from Uganda. However it is unclear whether this methodology is the right methodology to use. It was not a question that was subject to the PICO (problem, intervention, comparison, outcome) analysis used in the guidelines process, so some flexibility in interpretation should be considered. There may be other approaches that could be used to evaluate transmission, though it would be best for any approach used to be reviewed by WHO.

There were some details of the risk-stratified stop-MDA survey approach that would need input from the OTS. The maximum size of the assessment area (in terms of population or geographic area) needs to be defined. Additionally criteria are needed for determining when urban areas should be included in the survey.

Discussion: Much of the discussion focus on how to design a survey that made use of what is known about the epidemiology of onchocerciasis while also taking into account that unknowns remain. Specifically, it is known that the most intense transmission occurs near the breeding sites in what are called 1st-line villages. Although these villages are typically close to the breeding site and on the same side of the river as the breeding site, there are local factors that alter this pattern. It can be difficult to identify the location of all breeding sites that could be contributing to transmission, particularly in forest areas and low transmission areas where smaller streams and seasonal streams might harbor breeding sites. Also in some areas there are so many streams that nearly all villages are within 15 km of one. Additionally, programmes have imperfect knowledge of the breeding sites

in an area of transmission. Breeding sites can move seasonally and over time. Aspects of the transmission season may have changed as the timing of the rainy seasons has changed over time. Given the imperfect knowledge of the transmission dynamics of the entire area of transmission, methods are needed to address this. One possibility discussed was a modification of the proposed risk-stratified stop-MDA survey. The modified approach would adopt a 2-stage approach. In the first stage, purposive sampling would be used to evaluate the known 1st-line villages. In the second stage, random sampling would be used to evaluate the rest of the evaluation areas. It would need to be determined what portion of the sample should come from the 1st-line area and what portion should come from the rest of the evaluation area. A simpler approach would be to use the proposed risk-stratified approach but perhaps require minimum sample from the 1st-line villages.

WHO was asked to clarify what would happen, particularly in regard to verification of elimination, if programmes stopped MDA in an area according to previously proposed stop-MDA surveys. WHO responded that programmes are always encouraged to use WHO-recommended or supported approaches. However, if a programme area successfully completes a stop-MDA survey based on older methodology and then successfully completes PTS, its dossier for elimination should be accepted. It is the PTS evaluation that helps demonstrate that the decision to stop MDA was correct.

It was pointed out that including more vector assessments as part of routine M&E (during the treatment phase) could be helpful in improving the identification of 1st-line villages. It was also pointed out that the question of the optimal age-group for inclusion in stop-MDA surveys should be resolved**.** If the 5–14-year-old age group is used, it will be important to adjust sampling strategies to ensure that the age structure of the population is respected. It is typically easier to enrol the younger children. Including older children should increase the threshold for interruption of transmission. If younger children are over-represented and the threshold is increased, one could draw incorrect conclusions about the status of transmission.

The availability of blackfly data would be helpful for confirmation of serological thresholds suggested by models.

<u>OTS Recommendations:</u>

- Compare the 1st-line village approach to the random approach to determine if the risk-stratified or two-stage approach is necessary and determine what proportion of the sample should be obtained in 1st-line villages
- Encourage vector surveys to help with determination of potential new serologic thresholds for stopping MDA ----------END OF REPORT

## IV. Declarations of Interests from Committee Members and Invited Participants
## Committee Members – Temporary Advisors

| Name | Region | Country | Institution | Declarations of interest (*i.e.*, related to the topic of the meeting/guideline) | Meeting/ review restriction |
|---|---|---|---|---|---|
| Thomas Unnasch | WHO Region of the Americas | United States of America | University of South Florida | Has received funding for onchocerciasis related activities from the Mectizan Donation Programme and the Carter Center | None |
| Upendo Mwingira | WHO African Region | United Republic of Tanzania | Ministry of Health, Community Development, Gender, Elderly and Children | No | None |
| Ricardo Thompson | WHO African Region | Mozambique | Institute of Health | No | None |
| Katherine Gass | WHO Region of the Americas | United States of America | The Task Force for Global Health/NTD Support Center | Has received funding for onchocerciasis related activities from the Bill and Melinda Gates Foundation, the U.S. Agency for International Development, and the U.K. Department for International Development | None |
| Asam M.A. Zarroug | WHO Eastern Mediterranean Region | Sudan | National Program for Prevention of Blindness, Federal Ministry of Health | No | None |
| Robert Klein | WHO Region of the Americas | Guatemala | Universidad del Valle de Guatemala | No | None |
| Joseph Kamgno | WHO African Region | Cameroon | University of Yaoundé | No | None |

**WHO Participants**

| Name | Region | Country | Institution | Declarations of interest (*i.e.*, related to the topic of the meeting/guideline) | Meeting/Review restriction |
|---|---|---|---|---|---|
| Daniel Cohn | WHO Region of the Americas | United States of America | RTI International | Has received funding for onchocerciasis related activities from the U.S. Agency for International Development | None |
| Chukwu Okoronkwo | WHO African Region | Nigeria | Neglected Tropical Diseases, Public Health Department, Federal Ministry of Health | No | None |
| Bihran Mengistu Abtew | WHO African Region | Ethiopia | Neglected Tropical Diseases Officer, Federal Ministry of Health | No | None |
| Wilma Stolk | WHO European Region | Netherlands | University Medical Center Rotterdam | Has received funding for onchocerciasis related activities from the Bill and Melinda Gates Foundation, U.S. Agency for International Development, and the Malawi Ministry of Health | None |
| Daniel Adjei Boakye | WHO African Region | Ghana | University of Ghana | No | None |
| Sharon Roy | WHO Region of the Americas | United States of America | U.S. Centers for Disease Control and Prevention | No | None |
| Kimberly Won | WHO Region of the Americas | United States of America | U.S. Centers for Disease Control and Prevention | Has received funding for onchocerciasis related activities from the U.S. Agency for International Development | None |
| Allison Golden | WHO Region of the Americas | United States of America | PATH | Has received funding for onchocerciasis related activities from the Bill and Melinda Gates Foundation | None |