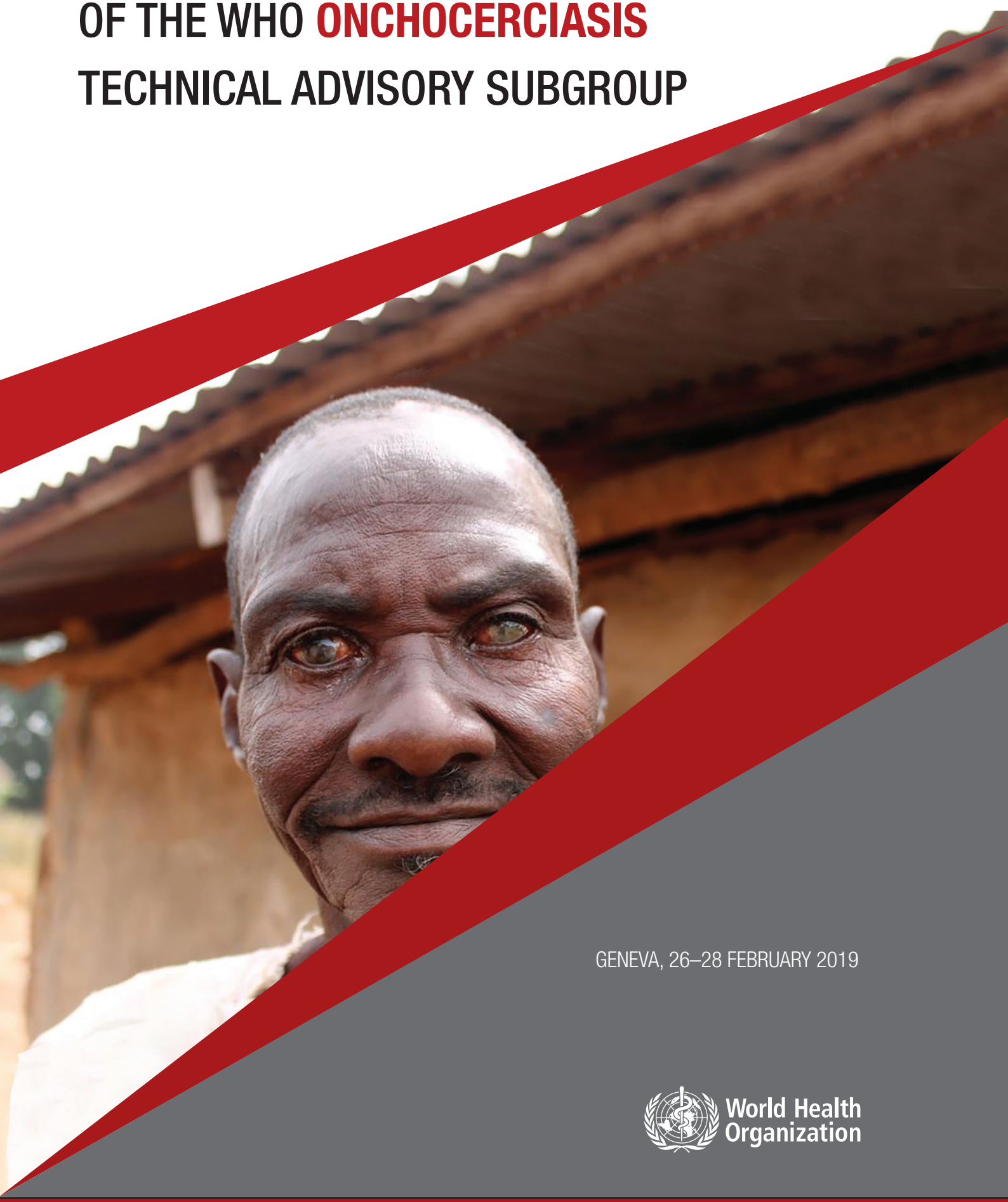
REPORT OF THE THIRD MEETING OF THE WHO ONCHOCERCIASIS TECHNICAL ADVISORY SUBGROUP



GENEVA, 26–28 FEBRUARY 2019

World Health Organization

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ISBN 978-92-4-000663-8 (electronic version)
ISBN 978-92-4-000664-5 (print version)

Printed in France.

Table of Contents

Abbreviations and acronyms

AP	alkaline phosphatase
APOC	African Programme for Onchocerciasis Control
CDC	United States Centers for Disease Control and Prevention
DBS	dried blood spots
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
HRP	horseradish peroxidase
LF	lymphatic filariasis
LGA	local government area
mAb	monoclonal antibody
MDA	mass drug administration
M&E	monitoring and evaluation
mf	microfilariae
NIH	United States National Institutes of Health
NOEC	national onchocerciasis expert committee
OD	optical density
OEC	onchocerciasis expert committee
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
TAS	transmission assessment survey
TPP	target product profile
WHO	World Health Organization

Executive summary

The third meeting of the Onchocerciasis Technical Advisory Subgroup (OTS) of the World Health Organization (WHO) Department of Control of Neglected Tropical Diseases' Monitoring and Evaluation Working Group was held at WHO headquarters in Geneva, Switzerland, on 26–28 February 2019. The meeting reviewed new data comparing the available serological platforms for diagnosis of onchocerciasis and new data related to onchocerciasis elimination mapping (OEM). Additionally, it reviewed and provided input to the development of milestones relevant to elimination of onchocerciasis (interruption of transmission) for the achievement of the 2030 Sustainable Development Goals.

1. Comparison of available diagnostics

Data from a variety of settings and countries were examined to determine programmatically relevant performance characteristics. The major comparisons included:

- the Standard Diagnostics (SD) Bioline enzyme-linked immunosorbent assay (ELISA) kit;
- the United States Centers for Disease Control and Prevention (CDC) adaption of the alkaline phosphatase (AP) ELISA used by programmes in the Region of the Americas and in Ethiopia, Nigeria, Sudan and Uganda; and
- the SD Bioline rapid diagnostic test (RDT).

The version of the ELISA used in the Region of the Americas and in the aforementioned countries is referred to as the Onchocerciasis Elimination Program of the Americas (OEPA) ELISA in this report. In addition to reviewing results from a variety of field settings, a multi-site laboratory comparison of the available diagnostics that involved laboratories in the United States of America, Cameroon and Kenya took place. The comparisons of the available tests were challenging given the lack of a gold standard diagnostic and the paucity of other diagnostic data (e.g. skin snip polymerase chain reaction (PCR) or black fly PCR results). Generally, the SD Bioline ELISA kit yielded more positive results than the AP ELISA, which generally yielded more positive results that the SD Bioline RDT. Few comparisons involved the OEPA ELISA. There remained concerns that the SD Bioline ELISA kit yielded positive results that were not programmatically relevant because the discrepancy between the kit and the RDT was so large in many settings, and the too few positive results were based on results in several settings that had AP ELISA or other ELISA results.

Although all of the tests have been evaluated previously with specificity panels and have shown to be highly specific, some unanticipated false–positive results were obtained in samples from areas in which onchocerciasis is not endemic. The programmatic relevance of this was unclear at this time; the OEPA ELISA platform has been used in both the Region of the Americas and in the aforementioned countries without the level of cross-reactivity in the evaluation presented.

Test performance is not the only factor in deciding which test to use: the cost of the test, the logistics of procurement, the ease of quality assurance and quality control (QA/QC), and the reproducibility and standardization of results are also important. Data are being collected on all of these important considerations so that an informed decision can eventually be made. Regardless of the challenges, the ELISA has been used in a variety of formats and settings to demonstrate the impact of ivermectin treatment, to identify programmatic areas that are performing well and not well and to meet the WHO criteria for stopping mass drug administration (MDA).

As many programmes wish to proceed with OEM) it was important to try to find a way forward. Experiments demonstrated improved performance of the SD Bioline Ov16 RDT using blood eluted from dried blood spots (DBS) rather than from whole blood collected in the field. Finally, preliminary data from a new dual antigen test strip for onchocerciasis were reviewed. The addition of a second antigen (OvOC3261) to a lateral flow assay allowed identification of skin snip positive individuals who were missed by anti-Ov16 antibody response.

Recommendations

- Insufficient data are available for a recommendation of one ELISA platform over another. Programmes should continue to use the ELISA that they prefer; a QA system should be implemented and those data shared transparently with national onchocerciasis elimination committees to ensure that their decisions are based on the best available data.
- For OEM, DBS should be collected for mapping. Programmes could proceed with mapping using ELISAs or RDTs performed using blood from eluted DBS in a laboratory setting. Additional work is needed to define the performance of RDTs using blood from eluted DBS and provide recommendations for appropriate QA/QC.
- Additional ELISAs should be compared, including additional comparisons of the OEPA ELISA, and more data collected on intra- and interlaboratory variability in results. The unexpected false–positive results should be explored further. Until then, countries should continue to use the ELISA they prefer to evaluate when to stop MDA. Robust QA/QC systems should be implemented.
- The preliminary results of the new dual antigen test strip are encouraging; new tests that are better suited to programmatic needs, as specified by WHO, should be developed as a priority.

2. Onchocerciasis elimination mapping

Data from a variety of pilot survey of first stage of OEM were discussed, and presentations were given on the statistical considerations that should be included in determining the target threshold for starting MDA.

Recommendations

1. Exclusion mapping (i.e. identification of unmapped areas where the environment is unfavourable for the presence of black flies) is important and should be undertaken before proceeding to stage 1 mapping.
2. Mapping should begin in high-risk areas (e.g. in areas near hyper- and meso- endemic districts or in areas where onchocerciasis was found during previous surveys). National programmes may wish to delay OEM in areas at lower risk until more data from pilot surveys become available.
3. Stage 1: Purposeful sampling of first-line villages
    a. Select five first-line (or high-risk) villages.
    b. Draw a convenience sample of 100 adults.
    c. Test using eluted DBS on RDTs if programmes have insufficient experience with ELISA; save left over DBS. If ELISA is used, the QA/QC procedures should be properly documented and the results recorded.
    d. If Ov16 prevalence in one or more villages exceeds the statistical threshold, then initiate MDA.
    e. If Ov16 prevalence in all sites is below the statistical threshold, then proceed to stage 2 as merited by context.
4. Stage 2: Random sampling of villages
    a. The recommendations for stage 2 sampling are for operational research purposes only at this time.
    b. Group villages by risk (e.g. first-line villages not included in stage 1 mapping, followed by second-line villages, followed by the remaining villages).
    c. Systematically select 20 villages from the grouped list of villages.
    d. Sample 50 adults per village using the Expanded Programme on Immunization ("random walk") sampling, with an effort made to achieve an equal balance of men and women.
    e. Test using eluted DBS on RDT or ELISA as described in 3c above.
    f. If Ov16 prevalence is ≥ 5% in **two or more** villages **or** ≥ 10% in one or more villages, then initiate MDA; this threshold should be adjusted based on the performance of the test used.

g. If the above criteria are not met, and there are no additional concerns, then no MDA is indicated.

5. If infection appears focal (i.e. positive villages are clustered in one area), subdistrict decisions can be made, and the survey area can be stratified post-hoc. However, in each stratum that is not targeted for treatment the total sample size should be completed by sampling additional villages to reach the full complement of 20 and the criteria for initiating MDA must be reapplied to that strata using data from all 20 villages. The national onchocerciasis expert committee (NOEC) or WHO should be consulted to review this information.
6. Additional research is needed to:
    a. assess the spatial correlation of Ov16 and O150 PCR in different ecological settings (e.g. savannah versus forest) to determine its applicability in hypo- endemic areas; and
    b. determine the appropriate Ov16 biological threshold (i.e. prevalence) that is consistent with the potential for ongoing transmission; entomological indicators should be part of this assessment.
7. There is no recommendation to combine mapping with transmission assessment surveys (TAS) at this time; however, such surveys should still be used to evaluate transmission and, if transmission is found, then ivermectin MDA should continue.

Several concepts should be emphasized. Although the concept of first-line villages (i.e. the village closest to a breeding site) is not new to national onchocerciasis elimination programmes, they should carefully consider how to identify these villages. Many programmes may be basing identification on historical understanding of the location and productivity of breeding sites. As these sites are not necessarily static, programmes should not rely on historical knowledge but should verify the current location and productivity of breeding sites in order to appropriately plan assessments related to stopping MDA and OEM activities. Efforts to identify breeding sites and/or verify the presence of black flies are important aspects of OEM. As data are still being gathered about OEM, programmes should focus their efforts on mapping those areas where transmission is most likely first. Until new, more efficient ways of determining that no transmission occurs in areas at very low-risk are developed, it may be a better use of resources to wait until additional data are gathered and analysed before conducting OEM in very low-risk areas. Countries should also carefully consider when OEM should occur at a subdistrict level. This requires considering the community-specific prevalence measured and the context (i.e. the results in nearby communities and the known status of transmission in neighbouring districts). Consultation with NOEC will be important in order to make this decision.

All of the thresholds developed and discussed in this document for OEM depend on the sensitivity and specificity of the test being used. Consequently, adjustments will need to be made once diagnostic performance is well understood. The appropriate method for second-stage OEM is still a matter of debate. The protocol presented should be considered a starting point for operational research. Alternatives to this strategy should be compared with this strategy. Additional research to confirm the biological threshold for transmission is recommended, with emphasis on the need to include entomological indicators of transmission.

3. Milestones for onchocerciasis elimination

Potential milestones were identified and discussed. Intermediate milestones for measuring impact (e.g. MDA stopped or completed post-treatment surveillance) are important to help countries and WHO track progress towards interruption of transmission. Emphasis could be given to a milestone for stopping MDA, to facilitate more rapid demonstration of progress and impact, or a milestone for completing post-treatment surveillance, to demonstrate impact more definitively. National programmes and WHO should be jointly accountable for achievement of the milestones. These suggestions were presented to WHO and subjected to additional input from a wide range of stakeholders.

1. Introduction

While the 2016 WHO guidelines for stopping mass drug administration (MDA) and verifying elimination of human onchocerciasis have provided important guidance to countries in which onchocerciasis is endemic, challenges remain, particularly with respect to activities that should take place in countries before implementation of these guidelines. Many WHO Member States endemic for onchocerciasis aim to eliminate the disease and seek expert advice on the way forward. The Onchocerciasis Technical Advisory Subgroup (OTS), an advisory group established in 2017, provides advice to WHO by developing and reviewing evidence to establish standard strategies and best practices and to inform the creation of new guidelines for the elimination of onchocerciasis. The third meeting of the OTS addressed several key unresolved issues from the first meeting in March 2017, particularly with respect to onchocerciasis elimination mapping (OEM).

The specific objectives of the meeting were:

- to compare two Ov16 ELISA platforms, the United States Centers for Disease Control and Prevention (CDC) alkaline phosphatase (AP) enzyme-linked immunosorbent assay (ELISA) and Standard Diagnostic (SD) Bioline (horseradish peroxidase, or HRP) ELISA and establish recommendations for the use of these assays;
- to evaluate the performance of the monoplex and biplex rapid diagnostic tests (RDTs), establish the contexts in which RDTs can be used and identify ways to improve the RDT to expand its use;
- to review data from onchocerciasis elimination mapping (OEM) pilot surveys, arrive at consensus on a sampling approach and finalize the strategy for a manual; and
- to establish milestones in line with the 2030 Sustainable Development Goals and set target dates for those milestones.

2. Opening session

2.1 Summary of the second meeting

The chair presented a summary of the second meeting (Geneva, 12–14 February 2018), which focused on areas co-endemic for *Loa loa*. Key discussion points were as follows.

i. The Loascope performed well, although a question remains about variability in the timing of peak microfilaremia. This is a possible question for operational research. Additionally, it is important to determine cases in individuals who were tested and treated the previous year and whether it is reasonable to re-treat them without additional testing. Otherwise, the Loascope would need to be used on everybody every year. Compliance with MDA in an area that had used the Loascope for two consecutive years increased from 60% to 70%.

ii. The Test and Not Treat strategy has a no-treat cutoff of 20 000 microfilariae (mf)/mL blood, which is very conservative given that the lowest load associated with severe adverse events (SAE) in studies is 50 000 mf/mL. On whether it was ethical to subject people in loaiasis-endemic areas to a risk of *any* SAE in hypo- endemic onchocerciasis areas where an individual's benefit from ivermectin is minimal with respect to onchocerciasis morbidity and the ancillary benefits of treatment, do these benefits balance out the risk of SAEs? A proposal was made to convene a WHO ethical sub-committee to discuss this.

iii. Modelling data were presented. There are significant differences between the ONCHOSIM and EPIONCHO models, particularly in risk of recrudescence, but both models predict a slow return of parasites is possible, suggesting a 3-year post-treatment surveillance (PTS) timeframe may be too short, and a post-elimination (PES) framework is needed. Of note, local vector biology and transmission dynamics vary greatly and may affect the dynamics of recrudescence.

iv. Central laboratory – a central reference laboratory is important for quality control (QC) and quality assurance (QA), but local laboratory capacity is important for processing the many

samples that are and will be produced. Developing an accreditation process will be important in this context.

v. Entomology – there is a need for increased local capacity, especially at the field level. The OTS recommended that programmes begin entomological work (to identify breeding sites, biting rates, etc.) well before this information is needed to demonstrate interruption of transmission. Polymerase chain reaction (PCR) is not necessary in these exploratory studies. Standard manuals need to be updated and need to emphasize that the WHO guidelines represent a *minimum* requirement; countries can always do more.

vi. Lymphatic filariasis (LF) and onchocerciasis co-endemic areas – the OTS recommended integrated evaluations to the extent possible: adding onchocerciasis to LF transmission assessment surveys (TAS) in order to obtain monitoring and evaluation data for onchocerciasis. The question was raised about how to conduct OEM surveys in areas getting ivermectin for LF (this was discussed further at this, the third meeting).

vii. Key conclusions of the first meeting:

- Rapid epidemiological mapping of onchocerciasis (REMO) is not suitable for mapping hypo- endemic areas.
- An onchocerciasis elimination mapping algorithm for pilot studies was agreed upon.
- Exclusion mapping to eliminate areas not suitable for onchocerciasis transmission will reduce mapping needs.
- The appropriate mapping unit should be identified based on local expertise.
- The general approach is to identify first-line villages for initial screening; if these sites fail, then treat; if these sites pass, go to random villages. For mapping, the 0–5 years age cohort is not informative, and it is better to use adults.

viii. Unresolved issues on onchocerciasis elimination mapping from the first OTS (addressed at this third meeting):

1. How many first-line villages should be evaluated?
2. What is the process for selecting random villages? How many should be screened? How many individuals per village should be screened?
3. What is the threshold for treating/not treating?
4. Which assay should be used: RDT or ELISA? If ELISA, which ELISA platform?

2.2 Review of serological tests

Presentation: At its first meeting in March 2017, the OTS recognized the need to standardize Ov16 serology and recommended evaluations of ELISA formats in multiple laboratories. Three established laboratories – the US CDC (Atlanta, GA, USA), the Centre for Research on Filariasis and other Tropical Diseases (CRFilMT) (Yaoundé, Cameroon) and the Kenya Medical Research Institute (KEMRI) (Nairobi, Kenya) participated in these comparisons. Additionally, other existing and newly developed tools were also evaluated in these locations to generate a comprehensive summary of the performance of available diagnostic tools for onchocerciasis. The main assays reviewed were the SD ELISA, the CDC AP ELISA, the SD Onchocerciasis IgG4 monoplex lateral flow assay (RDT), the SD Onchocerciasis/LF biplex lateral flow assay and a newly developed prototype dual antigen (Ov16/OC3261) test strip that is not commercially available. The SD ELISA used for the evaluations was a pre-commercial kit produced by the manufacturer; plates and critical reagents were packaged in the kit. In contrast, the CDC AP ELISA required separate procurement of each of the necessary components. The SD monoplex and biplex were purchased directly from the manufacturer. The dual antigen test strip prototype used is not currently available for programmatic use but was developed at the United States National Institutes of Health (NIH) and produced at PATH specifically for this comparison. The objective of these comparisons was to determine which onchocerciasis platform(s) should be recommended for programme use. Several factors were considered collectively when assessing the most appropriate tool(s) for mapping, monitoring and stopping MDA. Elements considered included the assay platform and performance characteristics, type of personnel needed to

perform each assay, where the assays can be conducted, sample type needed, time needed to obtain a result, ease of use and cost. Ideally, each aspect of the laboratory analysis should be as standardized as possible. The RDT can be run using whole blood or blood eluted from dried blood spots (DBS). In comparing assays, cost, logistics, feasibility, throughput and assay performance are all factors that affect the selection of the best assay. Advantages of the SD ELISA include fewer import restrictions (fewer items to import) and higher throughput (2.5 hours processing time for the SD ELISA versus 6.5 hours for the AP ELISA, not including overnight elution of the DBS). The SD ELISA is more sensitive than the AP ELISA, which in turn is more sensitive than the RDT, which means greater discriminatory ability with the SD ELISA, especially at lower concentrations of antibody in the sample.

3. Country presentations

3.1 Mali: nearing stop MDA

Presentation: Baseline mapping in the south of Mali in the 1970s to 1980s revealed a microfilarial prevalence > 60% in several districts and prevalence of 30–60% in the majority of districts. Through vector control and over 30 years of ivermectin distribution, the prevalence was dramatically reduced; only two districts have a skin snip prevalence of 0.1–5%. Twenty districts are currently under ivermectin MDA, mostly for LF. An onchocerciasis expert committee (OEC) was created in 2016, and in 2018 a strategic plan for onchocerciasis elimination was written. Priority actions include updating the map of breeding sites, OEM where indicated, and pre-stop and stop MDA assessments. To date they have identified districts that may need OEM and pre-stop MDA assessments. Country constraints and challenges include limited capacity for field and laboratory work, risk of cross-border transmission and the fact that although the country has a lot of data much of it does not meet WHO criteria for making stop-MDA decisions.

Discussion: There needs to be a way to capture the complexity of the data: number of sites, number of people tested per site, etc., to allow better interpretation of the results. There was a suggestion to generate a template to standardize the collection and presentation of surveillance and other data across countries. It was noted that there are cross-border issues with Burkina Faso, Guinea and Côte d'Ivoire. A recent PLoS publication looks at the focus near the border with Senegal. The country received support to develop their elimination plan, and it should probably be finalized by now.

3.2 United Republic of Tanzania: nearing stop MDA

Presentation: Of the 55 million population in the United Republic of Tanzania, 6 million people are at risk of onchocerciasis in eight foci spread across 28 districts; all districts are co-endemic for LF, but 23 of them have already stopped LF MDA. Data from the Tukuyu focus were presented; Tukuyu focus comprises four districts with a population of 800 000 people and has received 18 annual rounds of ivermectin MDA. Baseline nodule prevalence was 5–58%. Skin snip microscopy surveys in 2011 and 2012 showed 0% mf prevalence in 1861 and 3146 people, respectively. Treatment coverage has been near but rarely at or above 80%. In 2015, a CDC/African Field Epidemiology Network study looked at different markers of infection – RDT (in central laboratory), ELISA and entomology – in 11 villages near known breeding sites. In each of five age groups (0–5, 6–10, 11–15, 16–20, and > 20 years), 200 people were enrolled. Each participant had blood drawn for RDT, DBS for ELISA, blood smears, and ICT (for LF), and two skin snips for microscopy and PCR. Flies were collected from corresponding breeding sites. Later, RDT using DBS and SD ELISA were added. Results by RDT, RDT using eluted DBS, biplex RDT using eluted DBS, AP ELISA and SD ELISA performed comparably in most age groups. In the > 20-year-old age group, RDT using heparinized whole-blood gave a significantly lower prevalence estimate than the other assays. Nearly 9000 flies were caught using human landing catches. All flies tested negative by poolscreen PCR. In 2016, a monitoring survey was conducted; a systematic sample of 332 children aged 6 to 9 years in 10 villages were tested by Ov16 RDT with DBS collected for later ELISA. All children were negative by RDT. Two villages were negative by SD ELISA: one had > 20% prevalence and the rest about 5% prevalence. Based on the results of the negative 2015

entomology and the 0% RDT prevalence in 2016, a full stop MDA evaluation was conducted in 2018. Probability proportional to estimated size (PPES) sampling was used to select 51 villages, and households were systematically sampled in each village to yield a sample size of at least 3000 children aged 6–9 years. Children were tested by Ov16 RDT on site and DBS were collected for later testing by SD ELISA. One (0.3%) of 3281 children tested positive by RDT and 323 (9.8%) tested positive by Ov16 SD ELISA. Since this last assessment, two more rounds of MDA have been completed.

Discussion: The serological results were a bit difficult to interpret. In general, the ELISAs produced more positive results than the RDTs, but the differences were not consistent across age groups. Follow-up studies demonstrated differences between the RDT and the AP ELISA and striking differences between RDT and SD ELISA results which are difficult to interpret. The prevalence of infection by field RDT, as has been seen in other studies, was much lower than the other testing formats.

3.3 Togo: nearing stop MDA

Presentation: Of the 40 districts in Togo, 32 are endemic for onchocerciasis. Baseline mf prevalence in the 1970s was as high as 83%. Vector control in the 1970s and 1980s followed by ivermectin distribution for the past 21 years has dramatically reduced onchocerciasis prevalence. Togo conducted a survey with CDC/African Field Epidemiology Network in two districts, Kozah (in 2016) and Yoto (in 2017), using the same methodology as in the United Republic of Tanzania. Both districts were hyperendemic at baseline. Kozah has received twice yearly MDA and Yoto has received annual MDA since 1988. In 2012, Kozah had mf prevalence by skin snip microscopy of 0.1–1% and Yoto had mf prevalence of 0.2%. Twelve villages were selected in each district and participants were selected and tested using a process similar to that of the Tanzanian study. Black flies were captured at two sites in each district and were tested both in Togo (by O-150 poolscreen PCR-ELISA) and in CDC in Atlanta (by O-150 poolscreen PCR). The relationship between RDT and AP ELISA was slightly different in the two sites. In Kozah, RDT results were below 1% prevalence until the > 20-year-old age group, when prevalence rose to 11.7%. The AP ELISA results were much higher than the RDT results, increasing from 0.5% in the youngest age group to 5.7% in the 16–20-year-old group, then rising to 31.5% in the > 20-year-old group. Conversely, in Yoto, the RDT and ELISA results were very similar except in the oldest group, where prevalence was 9.2% and 16.0%, respectively. Comparing results from Kozah by RDT, RDT on eluted DBS, AP ELISA and SD ELISA, the sensitivity increased from the RDT (lowest sensitivity) to the SD ELISA (greatest sensitivity). In the 6–10-year-old age group, seropositivity increased five-fold in the AP ELISA compared with the RDT and by 10-fold in the SD ELISA compared with RDT. Despite these differences, both districts yielded age–prevalence curves that reflect the 20-year history of treatment with ivermectin.

Togo presented data from a school-based survey comparing Ov16 RDT (on finger-stick whole blood in the field) to the PATH Ov16 ELISA (the HRP ELISA, which evolved into the SD ELISA kit). A convenience sample of 15 children aged 6–9 years was selected at two schools in every subdistrict in nine districts; 2654 children had RDT and ELISA results. A mixture model was used to set ELISA cut-offs: 0.5% of children were positive by RDT read at 20 min (the standard at that time), 2.8% were positive by RDT read at about 24 h and 8.1% were positive by PATH ELISA. The sensitivity of the ELISA was much greater than that for the RDT; concordance between RDT and ELISA was poor. Samples that were negative by RDT at 20 min and positive by RDT at 24 h were mostly negative by ELISA.

Discussion: Despite their differences, all the tests demonstrated the impact of ivermectin treatment and the programme was able to identify areas that were not performing well. However, there was poor concordance between the RDT and both ELISAs in Kozah and in the school-based study, though better agreement in Yoto. Generally, performing RDTs with DBS increased the positivity rate compared with RDTs performed in the field. As the RDT and ELISA results were more similar in Yoto than in Kozah, the question arose whether there was a systematic difference in the two settings. The field teams were the same, but it was uncertain whether the lot of RDTs was the same. The RDT lots were different in

Kozah and Yoto because some RDTs were loaned to the Kozah site from another activity, whereas the RDTs ordered specifically for this survey were used in Yoto.

3.4 Malawi: elimination mapping and nearing stop MDA studies

Presentation: The national onchocerciasis elimination programme started in 1984 in partnership with the Tea Association of Malawi. With support from the African Programme for Onchocerciasis Control (APOC), the country conducted rapid epidemiological mapping of onchocerciasis (REMO) in 1997, and it was established that onchocerciasis was hyper- and meso-endemic in five other districts in addition to the three previously known. MDA using community-directed treatment with ivermectin started in three districts in 1997 and in 2000 the MDA was extended to the other five districts. The OEM survey was conducted in Malawi in the context of onchocerciasis elimination to identify all untreated areas in the country where transmission of onchocerciasis might be ongoing. In three districts (Chitipa, Karonga and Dedza) that have never been treated for onchocerciasis, two had previously received four years of ivermectin and albendazole for LF but are now under post-MDA surveillance for LF; the other district has never received ivermectin. Two of the districts border endemic districts in the United Republic of Tanzania, and the third district is suitable for onchocerciasis transmission but had never been assessed. From an entomological perspective, there was no black fly biting reported and prospection for breeding sites indicated no breeding of *Simulium damnosum* at the time of evaluation.

The survey in 2018 used a list of all villages in the three districts as a sampling frame. The three sampling methods used were: (i) purposive sampling of four communities with high prevalence (first-line villages) based on the 1997 REMO mapping (in each village, 100 people aged 5–9 years, 100 people aged 10–19 years and 100 people aged ≥ 20 years); (ii) PPES sampling of 30 communities in each district without regard to onchocerciasis risk (within each site a convenience sample of 50 adults aged 20 years and older was obtained); (iii) random sampling of 50 children aged 10–14 years from each of 30 schools associated with the 30 communities in (ii). All individuals were tested by biplex Wb-123/Ov16 RDT (using whole blood) and SD Ov16 ELISA (using DBS). Onchocerciasis prevalence by RDT was very low, ELISA prevalence was higher and prevalence in males was higher than in females. In all three districts, the random villages yielded higher maximum prevalence than the first-line villages. Mean Ov16 RDT prevalence in randomly selected villages was 0.1%, 0.1% and 0.6% in Chitipa, Karonga and Dedza districts, and mean ELISA prevalence was 5%, 9% and 7% in these same districts, respectively. Accessibility was one of the challenges experienced during the survey. The Malawi OEC will guide the programme during its May 2019 meeting on the way forward based on the results.

Discussion: The discussion focused on the identification of first-line villages, which were selected on the basis of the results of the 1997 REMO mapping. Nevertheless, there were no reports of black flies in these areas and, in the absence of any history of black flies, it is difficult to interpret the entomologic data. The survey teams conducted prospection for *S. damnosum* breeding sites but found none. Dr Boakye pointed out that the timing of looking for breeding sites is critical to actually finding them, as they may dry up or flood at certain times of the year. The determination of first-line villages is clearly essential to implementation and interpretation of elimination mapping activities. This topic was revisited later in the meeting, during the discussion of elimination mapping methodology.

3.5 Nigeria: mapping plus a question of thresholds

Presentation: This presentation described onchocerciasis elimination mapping in Wammako and Aleiro, two rural local government areas (LGAs) in the Sahel with baseline REMO nodule rates of 2% and 4–6%, respectively. There were no previous reports of black fly nuisance biting in either LGA; Wammako had four potential breeding sites and Aleiro had three, but black fly larvae were not found at any site at the time of inspection. The first-line villages selected for stage 1 sampling were 5 km from suspected potential breeding sites (i.e. rocky substrate and rapids); flies were not identified because the study was not performed during the correct season. In each LGA, first-line villages were selected by a combination of desk review, identification of villages within 5 km of the rivers and breeding site prospection. In each

LGA, three first-line villages were selected and in each village a convenience sample of 300 adults aged ≥ 20 years and resident in the village for > 10 years was selected and tested by RDT and SD ELISA. All RDTs were negative in all three villages in Wammako LGA, while one village had one RDT positive individual in Aleiro LGA. First-line village prevalence ranged from 2% to 9% by SD ELISA in Wammako. For stage 2 sampling, 30 villages in each LGA were randomly selected and 50 adults per village were tested. No positives were found by RDT in any randomly selected village in Wammako LGA; five positives were identified from five villages in Aleiro LGA. In Wammako LGA, random village prevalence by SD ELISA ranged from 0% to 16.3%; Aleiro LGA SD ELISA data are still being analysed. Mean prevalence of stage 2 villages in Wammako LGA was 3.2% by ELISA and 0.0% by RDT. In Wammako LGA, the highest village prevalence was found in a random village, but all (3/3) first-line villages had at least one positive, whereas only 2/3 of random villages had at least one positive test. Based on the SD ELISA data, Wammako qualifies for MDA; a final decision will be made with the NOEC in May 2019. Six teams of three people took 6 days to complete the OEM field work in one LGA; it took one day to complete sampling in each village (whether first- line or randomly selected). Challenges included: identification of breeding sites; the breeding sites are seasonal and most of the rivers had dried up at the time of prospection; the Expanded Special Project for Neglected Tropical Diseases electronic data collection app had issues and crashed frequently on the older phones available for field work. The implementation team recommends the use of pre- and post-tests when training field workers to identify areas of weakness among the trainees, as this worked well when carried out as part of the Nigeria training.

Discussion: It is important to do RDT and lot QC to make sure that the RDTs are working. In the work presented from Nigeria, daily QC checks were done in accordance with the recommended protocols. All RDTs and lots passed the daily QC checks. Despite this, *all* RDTs were negative, but given that the specificity of the assay is not 100% we would expect to see some false–positives.

3.6 Ghana: mapping

Presentation: The Ghana Health Services onchocerciasis programme began in 1974 with aerial larviciding; treatment with ivermectin was progressively added through mobile vans and community-directed treatment, with the addition of intensified treatment in special intervention zones; REMO mapping was conducted in 2008. Considerable entomological work has been done in Ghana, including a large black fly breeding site prospection activity in 2017. A nationwide onchocerciasis impact assessment was launched also in 2017. A separate OEM exercise was conducted in Asunafo North and Dormaa West districts using the two-tiered OEM sampling methodology described previously. In the four first-line villages in Asunafo North district, RDT prevalence ranged from 6% to 8.6% and SD ELISA prevalence from 5.6% to 20%. In the three first-line villages in Dormaa West district, RDT prevalence ranged from 1% to 3% and SD ELISA prevalence from 4% to 11%. In both districts, all of the first line villages exceeded the 2% treatment threshold by ELISA; one first- line village in Dormaa West did not exceed the 2% treatment threshold by RDT. In Asunafo North, 24 of the 30 randomly selected villages exceeded the treatment threshold by RDT and 29 by SD ELISA. In Dormaa West, half of the randomly selected villages exceeded the treatment threshold by RDT and 28 by SD ELISA. Aspects of the survey that worked well included appointment of a dedicated logistician/QC officer, use of WhatsApp for communication and coordination in the field, daily meetings of teams with supervisors to review challenges and issues, and the involvement of local staff to mobilize communities which facilitated high community participation. There were issues with bar code scanning; software did not detect previously scanned QR codes and this led to some errors. participant forms were difficult to edit if a registered participant later decided to opt out. Duplicate QR codes were created by both the manufacturer and the enroller. Finally, there were some issues with data entry and there was no way to edit errors out of entered data. Other challenges included a lack of Ov16 RDT controls. The Ov16 RDT kits ordered for the study arrived in country and cleared customs in 13.5 weeks; fieldwork had begun before their arrival. Recommendations include: data collection software should be updated to detect and reject information already entered or scanned; data recorders should be able to edit information that is incorrectly entered; the access code for the server should be shared with the NTD Programme; manufacturer of QR codes should validate all codes to prevent duplicates; there were difficulties in

recruiting equal numbers of males and females and the OEM protocol should mention how to address this; innovative approaches to numbering and selecting houses should be developed.

Discussion: Much of the discussion centred around identification of breeding sites. It was suggested that satellite imagery, which has been used in some settings to identify breeding sites, might be a good way to identify first- line villages but would require validation, particularly in forest areas. This might be a good approach in instances to help identify breeding sites. The Institute for Health Metrics and Evaluation used environmental data to generate a predicted onchocerciasis suitability index, which could help programmes prioritize areas that may be more suitable for black fly breeding; however, the index does not help identify breeding sites.

3.7 Kenya: mapping

Presentation: The OEM operational research protocol was conducted in six sub-counties in western Kenya that may have been historically endemic but the vectors were believed to have been eliminated in the 1950s and 1960s due to DDT and agricultural chemicals. Four first-line villages were selected from each district for stage 1 sampling, based on proximity to fast- flowing rivers, presence of symptomatic residents, historical information about infection and proximity to known endemic areas across the border. In each district, 30 randomly chosen villages were selected for stage 2 sampling. As part of the operational research, 30 schools matched with the location of the randomly chosen villages were selected, and children aged 10–14 were sampled. Overall, the Ov16 RDT prevalence was very low. Just two Ov16 RDT positives were found in one first-line site. Among randomly sampled villages, the Ov16 RDT prevalence was similarly low, with subcounty average prevalence ranging from 0% to 0.2%. The most surprising finding was that in Mount Elgon subcounty a total of six Ov16 RDT-positive children were found in the school sampling (four children from a single school), whereas only one Ov16- positive adult was found in Mount Elgon. SD ELISA analysis is planned.

The logistics of the mapping were challenging. It took up to 6 days to complete mapping activities in a first-line village and up to 3 days to complete activities in a randomly selected village plus matched school. In this study, testing was done onsite in the household, rather than in a central location. There were also some issues with communication and coordination of the activity. Questionnaires given to the study personnel highlight the need for good community sensitization and mobilization.

3.8 Ethiopia: a question of thresholds

Presentation: The OEM operational research protocol was conducted in seven woredas (districts) in Ethiopia, using the previously described methodology plus the addition of matched schools. Random sampling within villages was greatly complicated by the lack of village (sub-kebele) level population information at the central level. In two woredas, Ov16 RDT was negative in all first-line villages. In the other five woredas, as least one village had positive results, with prevalence ranging from 1% to 11%. Three woredas had results by RDT below the provisional threshold. Prevalence in men was more than twice that of women. There was a trend of increasing Ov16 prevalence with age (up to 40 years). In the randomly chosen sites, the mean prevalence was 0–2.6%; however, the individual village results varied greatly, with some sites having 10–20% Ov16 RDT prevalence. In two woredas, the results from random testing indicated a need for MDA, whereas the first-line village testing did not. The presence of black flies was reported in nearly half of all villages sampled. The random sampling appears to have identified higher prevalence villages than the first-line village sampling, and maps of the data suggest clustering of infected villages. The SD ELISA has not yet been run on these specimens, but it is planned.

This evaluation took one day per village to conduct the field work for first-line villages and one day per village for random villages. Blood was collected into tubes and, in most but not all areas, testing was done in a central location. There were some unexpected delays in getting the RDTs through customs and some challenges in accessing some of the remote villages.

3.9 Burundi: mapping

Presentation: Based on REMO mapping, 35 districts are either hypo- endemic or of unknown onchocerciasis status. The mapping survey presented was conducted in June–July 2018 in three districts: Cankuzo, Mutaho and Nyanza-Lac. Cankuzo had no REMO data and no known breeding sites. Mutaho and Nyanza-Lac had nodule prevalence from 2.5% to 19% in surveys conducted during 2001–2013. All three districts were ivermectin- naive. Three rural villages with high risk of transmission based on REMO data were selected. Breeding site prospection was not done. In each village, 300 people were randomly selected, 100 each in three age groups: 5–9 years, 10–19 years and 20+ years, with a target of equal numbers of females and males. For the selection of the random villages, villages, households and individuals were sampled as described previously, with the addition of matched schools. By RDT, one district exceeded the threshold for MDA based on the first-line village results. SD ELSA prevalence was 4–10 times higher, and all three districts exceeded the threshold for MDA. For the random approach, one district exceeded the threshold for MDA by RDT, and all three exceeded the threshold by SD ELISA. Results averaged over the district were around four times higher by SD ELISA than by RDT. Comparing first-line and random villages, by RDT, one district exceeded the threshold for MDA by first-line village approach and had villages > 2% by random approach. By SD ELISA, all districts exceeded the threshold for the first-line village approach; the highest village prevalence was also in a non-first-line village. Comparing school and village ELISA prevalence, sometimes school prevalence was higher than the prevalence in the paired community, and sometimes prevalence was higher in the community (see also the discussion of school versus community data in the discussion section of 5.7: Onchocerciasis mapping in areas co-endemic for LF). Maps of the results showing subdistrict units or villages suggested some focality of results. Burundi plans to map additional areas and perform entomological studies.

Teams took one day to map a first-line village and one day to map a random village plus its school. Conducting the household census was the biggest challenge, but, fortunately, a malaria net distribution programme had done it previously and the teams drew on that census.

4. Comparison of ELISA platforms and RDT

4.1 ELISA verification phase and QA for serological tests

Presentation: The topics for this presentation included in-laboratory SD ELISA verification, stability testing of RDT and ELISA controls prepared by PATH, update on stress stability of SD ELISA and results of DBS elution methodology with respect to blood volume capacity of different papers used. Background was presented on the SD ELISA verification prior to use in field settings. PATH tested the verification lot RETC004 of the SD Ov16 ELISA with a panel of both serum/plasma samples and DBS. The DBS were prepared in the laboratory, combining washed red blood cells and archived matched sera/plasmas to yield a whole blood sample. The final panel consisted of 306 serum/plasma samples and 148 matched DBS. The average optical density (OD) at 450 nm of duplicate well results for each sample was normalized to the calibrator control included with the ELISA kit. A threshold was applied that would result in 100% specificity with the panels tested, or 0.52 normalized OD. The resulting performances were for plasma/serum and DBS, respectively: sensitivities of 86.9% and 74.3% and specificities of 100%. The same lot of ELISA kits was analysed by the laboratory at CDC and results combined with PATH in order to further analyse performance. A total of 667 total samples were run by PATH and CDC, 318 of which were DBS, some of which had been collected in field studies. PATH and CDC agreed upon 0.4 normalized OD as a modified threshold, with a measured combined sample (n=667) performance of 75% sensitivity and 99.5% specificity against reference assay of either microfilaria- or ELISA-positive. However, this threshold has been increased in response to further data from CDC, with most data analysed using 0.5 normalized OD threshold.

The precommercial activities that took place following the evaluation by PATH and CDC were to submit testing results to SD, for the Korean Ministry of Food and Drug Safety submission, internal QC

and stability testing, and production of requested pre-commercial lots. PATH conducted QC testing acceptable results for positive and negatives. It was noted that negative samples run from eluted DBS had slightly higher signal in the new lots as compared with the verification lot ETC001 kits sent to Smith College for training by CDC prior to in-country laboratory trainings. All passed criteria with PATH- and CDC-provided DBS. The higher negative signal noted with DBS prompted further testing of "problematic" negatives, showing that there may be lot-specific specificity differences using DBS between the ELISA kits. This issue has been raised with SD, and the manufacturer is open to both guidance on requirements and additional QC samples.

PATH conducted stability testing of the QA materials that have been made and sent by PATH to accompany the RDT (https://www.path.org/programs/diagnostics/dx-qa/) and, more recently, the SD ELISA. The controls are produced using a positive control monoclonal antibody (BioRad, P/N AbD19432_hIgG4) spiked into either human plasma or prepared as contrived DBS. For the human plasma controls, temperatures from -80 to 37 °C and daily cycling of 20–40 °C were tested for up to 16 weeks. Timepoints up to 6 weeks at all temperatures showed similar results on both SD ELISA and RDT. Conditions of -20 °C and 4 °C are stable at 16 weeks and likely longer. They were not impacted by volume loss. Volume loss in plasma controls was seen by 6 weeks at higher temperature and cycling, preventing testing beyond these timepoints. Cold chain shipment should not be necessary for QA materials, though longer storage at cold temperatures is recommended.

The SD ELISA has been tested in stress test procedures at SD. Accelerated testing was conducted on all individual kit components early in development. Components can withstand 30 degrees for 2 weeks. Further, shipping stability information is expected from SD. The current recommendation is controlled cold shipment, but ice-pack shipment may be feasible.

The effect of elution of DBS using different volumes and types of paper, when used in the SD ELISA, was shown. The three most-commonly used papers were found to hold different volumes of blood for a given 6 mm circle area. The blood volume:elution volume ratio used in the ELISA was modified to adapt the protocol for non-Tropbio DBS to equalize the signals resulting from different papers, either by adjusting the elution volume or the DBS area used.

Discussion: One discussion point was about providing QC for negative samples. This can be difficult because there needs to be the correct and representative group of negatives. Supplemental QC might be needed in the early days of use of the ELISA. It was also asked whether controls could be included in the SD ELISA kit. This could pose issues related to licensing and QC. Monitoring using external QC controls provides more robust monitoring of the process. The controls currently provided in the kit are not the same as the BioRad plasma positive controls developed at PATH.

Regarding the specificity of the assay, it was pointed out that the presence of IgG4 autoantibodies will mean that there are always false–positive results, so any effort to tune an ELISA to 100% specificity is not a reasonable goal. The PATH results included one positive result in an individual who was geographically excluded (i.e. not from an endemic area). Other ELISAs have identified potential false–positives in individuals with LF. It was suggested that consideration be given for inclusion of samples from people outside the onchocerciasis- endemic areas that have LF.

The cost per sample by test was estimated to be US$ 1.20 for monoplex RDT, US$ 1.80 for biplex RDT and US$ 100 per plate for SD ELISA (about 40 samples/plate). CDC has found that cost per sample run of the SD ELISA is on average US$ 5.34 (range: US$ 4.17–7.06), including the cost of cold chain. If controlled cold chain is demonstrated to be unnecessary, the cost of SD ELISA would be significantly reduced.

4.2 ELISA standardization

Presentation: A single ELISA protocol would be advantageous for the global onchocerciasis elimination programme because it would facilitate standardizing assay controls, data comparability, data interpretation, QC and training. Comparisons of two specific ELISA platforms, the SD ELISA and CDC AP ELISA, were undertaken in the US CDC, CRFilMT and KEMRI laboratories. Although there are technical differences in the standard operating procedures of these two ELISAs, controls were included that allowed comparable analysis of the two protocols within and among sites. In addition to the controls, samples from research studies conducted in Togo and the United Republic of Tanzania (in areas nearing elimination) were tested at CDC; samples from OEM surveys in Ghana were tested at CRFilMT; OEM samples from Malawi were tested at KEMRI.

A serum control used at a single dilution on all SD ELISA plates in Cameroon and Kenya performed similarly, indicating highly consistent kit performance and technician performance. Additionally, results from three controls included in the kit by the manufacturer and three concentrations of a humanized IgG4 monoclonal antibody (mAb) used as external controls performed as consistently as the serum control. Conversely, results from the same serum and mAb controls used on the CDC AP ELISA were quite variable. For the CDC AP ELISA, there were significant differences in performance of the controls both within and among the laboratories. Significant variability of controls on ELISAs can be an indication of inconsistent test performance, technician performance or both. In these settings, the same technicians performed both ELISA protocols. Consistent SD ELISA results reduced concerns that technicians did not have sufficient skills for conducting ELISAs.

Overall, relative sensitivity of the SD ELISA was higher compared with the CDC AP ELISA but varied across the sample sets tested. For all comparisons, a normalized cut- off of 0.5 was used as the positive threshold for the SD ELISA, and an OD value of 0.05 was used as the threshold for the CDC AP ELISA. For the Malawi OEM samples, tested at KEMRI, the proportion of positive samples by SD ELISA was approximately 16 times greater than by the CDC AP ELISA. For the Ghana OEM samples, tested at CRFilMT, the proportion of positive samples by the CDC AP ELISA was approximately 2.5 times greater than by the SD ELISA. Although the prevalence of Ov16 antibodies was higher by AP ELISA, 69% of the positive results were just above the positive threshold. For the Togolese and Tanzanian samples, tested at CDC, the proportion of positive samples by the SD ELISA was approximately 1.3 times greater than by the CDC AP ELISA. Unlike the OEM areas in Ghana and Malawi, the areas in Togo and the United Republic of Tanzania had received multiple rounds of MDA with ivermectin.

While the performance of the SD ELISA kit was highly consistent, there was some concern with the specificity of the assay. In the US CDC laboratory, a total of 320 DBS from individuals without onchocerciasis were tested by SD ELISA; onchocerciasis was excluded in all samples because they were collected in geographical areas where it is not transmitted. Of the 320 DBS, 239 were collected in five countries where lymphatic filariasis (LF) was endemic. The remaining 81 DBS were collected in two countries where, by geographical exclusion, neither onchocerciasis nor LF was present. Of the total tested, 34/320 (10.6%) were positive by the SD ELISA. Because sensitivity and specificity of the CDC AP ELISA had been established previously, the complete set of DBS was not tested by the AP ELISA. However, a subset of 78 DBS from three of the LF-endemic areas were tested to compare results. When tested on this platform, 34/78 (43.6%) were positive by the CDC AP ELISA compared with 11/78 (14.1%) by SD ELISA. Although a high proportion of the AP ELISA positive samples were very close to the positive threshold (0.05), there was a clear signal in approximately 20% of the samples. These results highlighted a potentially underappreciated specificity issue with the CDC AP ELISA.

A trained technician could comfortably test 640 samples per week by SD ELISA compared with 480 samples per week by the CDC AP ELISA. The difference was attributed to the total time needed to complete each assay (SD ELISA – approximately 2.5 h; CDC AP ELISA – approximately 6.5 h).

Logistical challenges existed for both ELISA formats. The SD ELISA required shipping with cold chain, adding significantly to cost. Efforts to eliminate the need for cold chain are under way in order

to reduce costs. The CDC AP ELISA required separate procurement of all critical reagents, including the Ov16 antigen. A shipment sent to Cameroon that contained all the supplies for the CDC AP ELISA was held in customs for more than 2 weeks, compromising the reagents. Although the cost of the supplies needed for the AP ELISA was lower than the SD ELISA, a high rate of repeat testing would reduce the cost savings. Additionally, because of the potential challenges of importation of multiple supplies from various sources, it is recommended to have all critical reagents packaged as a kit.

Cost for the SD ELISA for reagents and consumables only (no labour) ranges from US$ 4.17 to US$ 7.06 (average US$ 5.34); cost is very dependent on import fees. Cost for the AP ELISA is difficult to estimate, because each reagent must be acquired individually, and the cost does not include the cost of the antigen, which is currently provided free of charge. A formal cost analysis would be helpful.

Overall, technicians in all laboratories found the SD ELISA easier to perform, easier to obtain consistent results from and the preferred format between the two ELISAs. However, there is still a need to further investigate the nonspecific reactions observed on both platforms.

Discussion: There are important difference between the CDC and OEPA versions of the AP ELISA. The CDC uses a standard stop point (time) during the development at the end of the assay. The OEPA ELISA instructions are to read until a certain OD is reached for the most concentrated titre of the control curve, which can be difficult to standardize. The OEPA ELISA is also highly tuned to maximize specificity. In a meta-analysis of 69 000 DBS analysed using the OEPA ELISA, sensitivity was 50%, but the OEPA has been applied extensively in LF-endemic areas and no issues with false–positives have been observed. It would be good to see how the OEPA AP ELISA performs against the problematic samples.

Regarding consistency of results, with a relatively short period of training, laboratory technicians are able to perform the OEPA ELISA and, although more variable results are seen early on, consistency improves over time. It was noted that, overall, IgG4 Abs to Ov16 have performed quite well. Additional features that may affect the consistency of results were discussed. There was a recommendation to invest in an automated washer because consistency is better than with hand washing, but the Cameroonian and Kenyan laboratories performing the AP and SD ELISAs used hand washing and only had issues with consistency with the AP ELISA. Seeing an analysis of consistency of OEPA ELISA results would be helpful in understanding the differences between the three ELISAs.

4.3 Efforts to improve RDT: using DBS with RDT

Presentation: Previous studies had been conducted at PATH and CDC to examine the use of DBS on the SD monoplex and SD biplex rapid tests. Dried blood spots were eluted into a buffer and the eluted material was tested on the RDT. Results from these prior studies indicated increased sensitivity by this method compared with results from whole blood tested at the point of contact. The ability to collect just DBS in field surveys would impact field logistics, so this method was further evaluated by testing a subset of the Malawian DBS (n=1687) at KEMRI and subsets of the Tanzanian DBS (n=918) and Togolese DBS (n=994) at CDC. The elution buffer for the testing was provided by PATH, the SD biplex was used in both laboratories and the same protocol was followed at each location.

Table. Proportion of DBS- positives for Ov16 antibodies, by country and setting

Country	Setting	SD ELISA	CDC AP ELISA	DBS RDT
Malawi	OEM	33.0%	2.1%	1.5%
Togo	Stop MDA	11.4%	8.9%	5.3%
United Republic of Tanzania	Stop MDA	11.3%	8.47%	10.5%

It was determined that a single technician could process 1152 samples per week by this method.

Discussion: It was asked whether the RDTs were read at the standard time and whether results changed if they were read at 24 h. It was clarified that the RDTs were read at 1 h, in accordance with current recommendations. It was asked why there were so many positives by SD ELISA in Malawi. More ELISA positives were observed in districts co-endemic with LF, and there was discussion about nonspecific reactions on the SD ELISA related to LF. However, the answer is not clear.

Although this method to improve sensitivity was feasible, and there may be a role for using DBS on RDT in certain settings (to be examined further), the sensitivity of the DBS on RDT relative to either ELISA format was variable, so these results do not immediately obviate a programmatic need for an ELISA. It will also be important to evaluate the specificity of the RDT when used with DBS.

4.4 Efforts to improve RDT: dual antigen onchocerciasis test strip

Presentation: Based on screening of an *Onchocerca volvulus* protein array (described in Bennuru et al, 2016),[1] eight potential new diagnostic antigens were identified on the basis of preferential IgG4 reactivity of sera from *O. volvulus* mf+ individuals compared with appropriate control sera. From these eight, three (OvOC3261, OvOC12838 and OvOC5421) were selected for further assay development. Based on IgG4 reactivity to recombinantly expressed proteins (including Ov16, OvOC3261, OvOC12838 and OvOC5421) in a variety of immunoassay formats, OvOC3261 was identified as the protein that, when added to Ov16, could increase the sensitivity of IgG4-based assays to ~95% from the 70–80% sensitivity of Ov16 alone. OvOC3261 was next striped (in collaboration with PATH) along with Ov16 on a prototype lateral flow platform (biplex) and tested under laboratory conditions. Using reactivity to either Ov16 or OvOC3261 as the definition of seropositive, the new lateral flow prototype biplex performed well using ~395 sera (245 *O. volvulus* mf positive, 150 controls) showed a 98–99% specificity and a 94% sensitivity. Independent testing performed at the CDC using panels of mf+ and control sera (n= 282) showed similar results. These data were then used as the basis for production of ~10 000 strips to be tested under field conditions using eluted DBS.

Of note, OvOC3261 provides significant added sensitivity against mf+ Ov-infected subjects without compromise in specificity. There is a small subset of Ov-infected subjects who are negative to Ov16 and all three novel Ov antigens tested. In experimental infection of chimpanzees, appearance of IgG and IgG4 antibodies to OvOC3261 occurs later than for Ov16, with IgG appearing at, rather than before, the appearance of microfilariae. IgG4 responses to Ov16 and OvOC3261 diminish dramatically over time (5–10 years) following definitive treatment.

Discussion: The dual antigen test strip appears very promising and results of the field testing using eluted DBS will be interesting and important. It was asked whether there were studies to examine the potential for false–positives (e.g. from *Loa* areas). It was pointed out that probably most of the specificity issues are coming from the Ov16 and not the OvOC3261, because OvOC3261 is found *only* in *O. volvulus*. There were eight people who did not react to any of the four Ov antigens. Unfortunately, there is no unifying feature that provides an explanation for this.

4.5 Dual antigen test strip: additional data

Presentation: The development of a new dual antigen test strip allowed for its inclusion in the comparisons conducted in Kenya. Additionally, an evaluation of the dual antigen test strip was conducted at CDC using a panel of DBS (n=440) collected in various onchocerciasis-endemic and non-onchocerciasis endemic settings. In the CDC evaluation, a positive result on the dual antigen test was defined as positive by either antigen (Ov16 or OC3261) or both antigens. When a true positive was defined by patent infection (i.e. skin snip positive), sensitivity and specificity of the dual antigen test strip were 88.3% and 50.8%, respectively. When a true positive was defined by exposure (i.e. Ov16

[1] Bennuru S, Cotton JA, Ribeiro JMC, Grote A, Harsha B, Holroyd N, et al. Stage-specific transcriptome and proteome analyses of the filarial parasite *Onchocerca volvulus* and its *Wolbachia* endosymbiont. mBio. 2016;7(6):02028-16. doi:10.1128/mBio.02028-16; https://mbio.asm.org/content/7/6/e02028-16.

serology positive by the CDC AP ELISA), sensitivity and specificity were 90.0% and 94.6%, respectively. Seven DBS expected to be negative for antibodies to Ov16 and OvOC3261 were positive by dual antigen test strip. Of the five DBS samples Ov16 negative but OC3261 positive, four specimens came from the Philippines and one from Haiti. The remaining two DBS that were positive to both Ov16 and OvOC3261 were from the United Republic of Tanzania. Band intensity differed depending on the type of specimen used (serum versus DBS), with greater intensity observed when using serum specimens.

In the Kenyan laboratory, a subset of the Malawian OEM DBS (n=1687) was tested by the dual antigen test. The proportion positive based on test type is displayed in the table below.

Test	SD ELISA	CDC AP ELISA	DAS either*	DAS both**
Proportion positive	33.0%	2.1%	18.0%	7.3%

*Ov16 or OC3261 or both positive ** Ov16 and OC3261 both positive

Some false–positives were observed with the DBS in the CDC evaluation, both on the dual antigen strip and on the SD ELISA. In the Malawian OEM samples, the percentage positive was higher in the two LF-endemic districts than in the LF non-endemic district. This raises the question whether the dual antigen test strip and SD ELISA are cross- reacting with LF.

It was determined that a single technician could process 768 samples per week using the dual antigen test. While the throughput is higher than by ELISA and the sensitivity of this test was higher than the CDC AP ELISA, there is still a need to investigate underlying factors for nonspecific reactions observed when testing DBS.

Discussion: If a positive result on the dual antigen strip is defined as "either" antigen being positive, then this does boost sensitivity. Conversely, defining a positive result as "both" antigens positive should improve the specificity of the test. It is concerning that the DAS both category, which requires both Ov16 and OC3261 to be positive, finds a higher prevalence than the AP ELISA (or the RDT by DBS from the same sample set). This may be a result of the rapid manufacture of the strips but makes interpretation of these preliminary results a bit challenging. It was noted that due to time constraints, the OC3261 antigen was not purified to the fullest extent. If work continues with the antigen, it can be further purified to enhance the specificity of the dual antigen test strip.

4.6 Summary of RDT versus ELISA comparison

Presentation: This presentation summarized RDT and ELISA results and challenges, in follow-up to the presentation of the field testing of RDT and ELISA. The comparison of an assay to its reference can depend heavily on the set of samples tested. Two scenarios for ELISA anti-Ov16 intensity levels of an Ov-exposed population versus RDT were shown to demonstrate how the selection of the cut- off for negative and positive results will greatly affect its performance. One of the key questions is what intensity of antibody response is clinically relevant.

Concerns over existing assays that had been expressed thus far in the Day 1 meeting were presented one by one, and for each concern the reason for the concern, the contradictions to the concern and the potential ways to address the concern were discussed. Key concerns were as follows:

- The RDT used in the field point-of-care setting is viewed to be not sensitive enough. Some possible ways to address this are:
    - increase intensity of signal by lowering visible threshold;

    - add additional antigens (such as NIH antigen OC3261 in dual antigen strips) to address those non-reactive or weakly reactive to Ov16;
    - change the reaction parameters in the strip to increase sensitivity; and/or
    - evaluate the sensitivity in the context of the reference assay's specificity: compare results to entomological data to determine if the serology data are meaningful and representative of true positive.
- ELISA concerns related to the specificity of the SD ELISA and the CDC AP ELISA, and to the difficulty running the AP ELISA (whether CDC or OEPA version). Potential actions to address specificity issues include:
    - increase the threshold/positive cut-off (this should not be done without understanding the effect on sensitivity);
    - introduce supplemental QC at Standard Diagnostics;
    - understand the influence of the glutathione S-transferase (GST tag); and/or
    - compare with entomological data to determine if the serology data are meaningful.

  To address difficult use:
    - implement pre-diluted controls to include in the ELISA;
    - pre-coat plates;
    - determine best indicators for troubleshooting in absence of kit-included controls; and/or
    - increase the threshold/positive cut-off (as above).

An example of the ELISA results using a non-commercial non-SD ELISA method (PATH) was shown when GST alone vs Ov16-GST is used as the antigen. GST is a protein tag used in the purification of the Ov16 ELISA antigen for both the ELISA and the RDT that may potentially generate a false–positive result if there is binding of detection reagents to the GST tag of the antigen rather than to the Ov16 antigen. In two different settings, two different relationships were seen between the GST and the Ov16-GST ELISA. In Togo, some high GST-only values were filtered from the dataset due to uncertainty in the result. The number of affected samples was small, and the issue was manageable. In Cameroon, there were very high numbers of Ov16 ELISA positives, and the Ov16 ELISA OD values and GST OD values were highly correlated in a subset, particularly for samples with false RDT results. These data were very difficult to interpret; if high GST ELISA results could not be removed from the data set due to large numbers, the question becomes, what is a true positive in this context? This observation was meant to spur discussion and note that the reactivity to GST may be platform-specific and should be considered when next- generation diagnostics are being developed.

- Concerns over the DBS on RDT not being sensitive enough came from field sites and field laboratories, and these concerns were in contrast to previous reports that the DBS on RDT was a higher sensitivity method than whole blood run on the RDT.
- A subset of DBS tested at PATH using SD ELISA compared field RDT result and DBS on RDT results and showed higher sensitivity using the DBS on RDT for the subset (45% vs 79% compared with the SD ELISA). Additional results for the entire set could be produced if needed.
- Any changes to the RDT will affect this method, and this should be considered in the context of any proposed changes to RDT.

In summary, quality control of ELISA is needed in order for users to trust the results. Workflow improvement can be in the form of a kit, improvement of standard operating procedures and/or controls and materials. If there are important performance questions, a troubleshooting plan is needed to identify and answer the most important questions and identify actions to follow such answers. Consensus is needed among stakeholders on the appropriate panels to use to define true negatives and positives and whether they are relevant. Improvement of RDT for field use may be necessary. DBS on RDT is compelling in terms of resource efficiency – it is recommended to determine criteria for failure before abandoning. RDT improvements may improve this method as well. For all of the concerns listed,

comparison to non-Ov16 serology tests and non-serological data are needed and should be accessible for confirmation (such as entomology or serology with other antigens).

4.7 Diagnostics summary

Presentation: Evaluating diagnostic tests for programmatic use requires balancing performance, feasibility, logistics, throughput and costs. The performance of the tests evaluated identified challenges with each diagnostic. There were varying results from the field and laboratory evaluations of the existing diagnostic tests. Eluting DBS and testing by RDT gave varying results across laboratories; sensitivity was greater than or equal to sensitivity when using RDTs at the point of contact with whole blood. Variable performance of the AP ELISA will make it challenging to standardize across laboratories. Specificity of the SD ELISA is not adequate for programme decision- making. Further evaluation of the dual antigen test strip is needed before it can be used for programme decision- making.

There should be an accelerated effort to optimize existing tools for programme use. Common sample sets that can be used to investigate specificity issues with ELISAs and dual antigen test strip in a uniform manner should be identified. In the interim, programmes could collect DBS for testing at a later time, as refinements could take 1–2 years depending on the test and the change needed. When refining performance characteristics of the assays, it is critical to define the purpose of test (i.e. use case) and desired minimum acceptable test performance characteristics.

Discussion: It is critical to define the purpose of a test (i.e. its use case); the characteristics of the ideal test may vary depending on the phase of the programme. Rather than designing a programme around the available diagnostic, we need to think about the characteristics of a test that would suit the needs of the programmes. Programmes should be encouraged to collect DBS in case additional testing becomes needed moving forward. During the transition period, it is essential that programmes generate QA/QC data for whatever test they use and report data on the performance of the assay they use.

4.8 Discussion on ELISA and RDT

There was lively discussion around which test to support in which setting. Comments fell into several categories:

- test standardization and centralized testing;
- translation of results;
- "sufficient" versus perfect tests;
- use of entomology to confirm serology results;
- setting different thresholds for different tests; and
- development of a testing algorithm.

The discussion began with a general agreement that we need a test/approach that is comparable across sites. This ensures more test conformity and reduces variability of results across sites and users. One way to accomplish this would be to have the tests conducted in a centralized laboratory. There was concern that if a test is marketed as commercial, then there is the risk that end users will expect clarity around the results. Translation/interpretation of the results is very important. It also becomes more difficult to require that ELISA testing be done in a central laboratory if the test is perceived as "commercial". A commercialized test would have the advantages of easier standardization and simpler procedures for ordering supplies. Drawbacks would be cost and potential for delays in procurement.

Concerns were raised that results from the AP ELISA, SD ELISA, RDT and DBS on RDT are all quite different, and the relationship between the results of these assays is different in different settings. One person observed that despite significant differences in prevalence between the SD ELISA and RDT, in several countries the programmatic decision would be the same regardless of which test you use. In this

context, the concept of a "sufficient" test is important. It is important to note that what is considered sufficient will vary by context (mapping versus stopping MDA).

One suggestion was that different thresholds and sample sizes could be determined for each test. Although we do not yet have all the necessary data to do it, this option could be explored, particularly as more entomology results become available. It was noted that programmes do need information on how the tests are performing in order to interpret the results. If you know the parameters, at least the test is interpretable, even if the information provided by the test is not perfect. However, there needs to be appropriate QA/QC for this to be true. There may also be a need to do more entomology to determine how these tests are performing, by comparing prevalence of infectivity in flies with seroprevalence as determined by each of the serological tests.

There was an agreement to examine each use case, each situation in which a test is needed and discuss which test might be appropriate.

- Mapping – all agreed that DBS need to be collected for mapping at this point in time. If a programme is going to use RDT for mapping it should be run from DBS, as the sensitivity of the RDT from whole blood appears to be too low in low prevalence settings.
- Monitoring and evaluation (M&E) – there some agreement on using DBS for M&E. RDT alone could be used for M&E, but there will be a point where a more sensitive test is needed. There was some reluctance to say that the RDT should never be used at all. The DBS on RDT seems potentially promising, but there was concern that this is in fact considerably more complex than the field RDT. But the field RDT, again, does not have the sensitivity of the RDT from DBS in the laboratory.
- Stop MDA – for stop MDA we need DBS collected to be run on ELISA (but the differences between ELISA platforms still need to be resolved), so no decision was made about which ELISA should be recommended by WHO for development and deployment of QA and QC materials. Programmes could continue the ELISA that they have experience with as long as QA is performed, and that information is shared with their NOEC and is available for submission with the verification dossier.

The conversation moved to the idea of developing a testing guide, and all were supportive of this idea. Whatever the use case, and whatever the test performance characteristics, having an operationalizable guide is critical for ministries trying to move their onchocerciasis programmes forward while some of the issues discussed here are being resolved. The algorithm could also reflect the urgency of the need. What we recommend today does not negate the need to work on getting a better test or making a test work better in the field. In that vein, we could make recommendations for areas where a decision is needed now; in contexts where there is less urgency, the approach might be different.

Given the relative urgency of having a plan for mapping decisions, a proposal was made to use DBS eluted on RDT in a central (national) laboratory for mapping, possibly adding a portable reader to reduce operator variability. Although not all of the data that the committee would have wanted were available to make a final decision, a provisional recommendation to use DBS eluted on RDT was made, with further tests of the DBS eluted on RDT needed, particularly in laboratories in endemic areas. If low sensitivity is a concern, programmes with results above the threshold to start MDA could comfortably start treatment. Results below the threshold might require additional testing. It is important at this stage that programmes focus on mapping the highest risk areas, as these are the most likely areas to have transmission detectable by tests with lower sensitivity.

The discussion then turned to how to move this forward by identifying the missing data required to sort out the discrepancies. There were several approaches suggested.

- Entomology as confirmatory testing in whatever setting these serological tests are employed. It is something that can be done right now. Whether with mapping or stop MDA,

we want to interrupt transmission; if there is transmission occurring, testing flies should allow us to confirm it. One approach may be to return to those evaluation sites presented at this meeting where there appear to be some positives. Breeding sites should be identified, and flies tested. If there are no flies, then the serology results might be false–positives or indicative of historical exposure. Also, there was a proposal to ask communities about whether they have flies, but previous work suggests that relying on just a few people in a community to accurately report on black fly nuisance may be problematic and may not always correlate with testing results.

- A major issue remains the interpretation of negative results. Increasing sample size is always an option, but the OEM strategy uses a small sample size and the stop MDA sample size already requires 3000 children. Increases in sample size could be costly in terms of testing and personnel time. There was a proposal to increase the sample size to accommodate for the reduced sensitivity of the tests; however, this will also increase the number of false–positives as a result of the imperfect specificity. Consequently, sample size will need to take into account the performance characteristics of the given test. One proposal was to pool DBS like flies, to make a very large sample size easier to handle/process. Technically, pooling DBS would be possible, but there would be considerable work to standardize that approach.
- Simply proceed with DBS on RDT for mapping. If a place falls below the threshold for starting MDA you could simply not make a final decision until there is a better test or better guidance. This is predicated on the idea that DBS on RDT, if positive, will correctly indicate when treatment is needed but, if negative, may not be sufficient for definitively concluding that treatment is not needed. One concern about this is that if we move forward and recommend DBS on RDT, countries may quite often have to move to stage 2 sampling if they do not pick up positives in the first- line villages. This concern is in part due to the poor concordance between DBS RDT versus SD ELISA in laboratories in Malawi and the United Republic of Tanzania (concordance between the two tests was poorer than when performed at CDC).

The final proposal was to move forward with DBS on RDT, even if it is not perfect. If it is positive, treat; if it is negative, you have the option to do random sampling or wait until there is clearer guidance on the ELISA. All agreed that a clear way forward, with options, and with the implications of the different options clearly laid out, is the way to go. Then, the NOEC can look at the data in depth and weigh in to help guide countries in their decisions.

4.9 Recommendations on ELISA and RDT

1. Recommendations for test selection are based on the intended use: mapping, M&E and stop MDA.
2. For mapping, the recommendation is to use DBS on RDT in a country laboratory to allow programmes to move forward with mapping. Clear instructions on the elution protocol and how to obtain QA/QC samples and control are needed. Programmes with current ELISA capacity can continue to use the ELISA protocol with which they have experience. However, the QA/QC process used by the laboratory needs to be clearly documented. Details on the mapping algorithm are provided in the recommendations on onchocerciasis elimination mapping (section 5.8).
3. For M&E/surveillance, point-of- care RDTs may be fine as it is the trend in prevalence over time that is important, but there is support for eluting DBS onto RDT if countries want to boost the sensitivity of the RDT.
4. For stop MDA, use DBS on ELISA. No particular ELISA is recommended at this time; AP ELISA (CDC or OEPA) or SD ELISA kit are all acceptable at this time while additional evaluations are completed; however, the QA/QC procedures and data generated by the laboratory should be recorded and made available to NOEC upon request.

5. Entomology to determine the prevalence of infection in black flies in areas where discrepancies between the RDT, AP CDC ELISA and SD ELISA should be performed so that the data can be interpreted and thresholds for starting MDA and stopping MDA can be validated or refined.
6. Additional analysis of the AP OEPA ELISA should be provided so that its performance can be compared with the other two ELISAs (of particular interest is intra- and inter-laboratory variability in results).
7. It is a priority to determine the underlying explanation for the positive results by both the AP and the SD ELISA for specimens from non-endemic areas. Evaluating the AP OEPA ELISA and the RDT with these specimens could be informative.
8. A field-based comparison of diagnostics that includes skin snip PCR could help further examine the issue of potential false–positive ELISA results.
9. Development of new tests that are better suited for programmatic needs as specified by WHO should be a priority.

5. Onchocerciasis elimination mapping

This section of the meeting aimed to finalize the sampling methodology for onchocerciasis elimination mapping (OEM). The general approach, discussed and agreed upon at the previous meetings of the OTS, consists of two stages of sampling. The first stage is to sample first-line villages, where transmission is most likely to take place, followed by a second, random sampling of all villages in the district if none of the first-line villages exceed the threshold for starting MDA. Second stage sampling should occur in areas where there is concern that there may be transmission and where the environment is appropriate for transmission. The agreed-upon aspects of OEM were presented again to ensure full understanding of the logic and statistics behind the methodology, and to ensure consensus on the approach. The session began with a presentation on some field experience from OEM pilot projects.

5.1 Identification of first-line villages

Presentation: It is worthwhile for national programmes to invest significant effort into correctly identifying first-line villages. The benefits to the programme include: understanding the vector species and potential extent of the transmission focus, reducing the need for stage 2 sampling (by identifying the need for MDA in stage 1), identifying good sentinel first-line villages for programme monitoring, and identifying capture points for the entomological surveys required to stop MDA and during post-treatment surveillance (PTS). During the OEM operational research and pilot projects, programmes made different efforts to identify first-line villages. The results from the comparison of stage 1 and stage 2 suggest that, quite often, programmes do not inherently know where the best first-line villages are. While some of the early adopter countries used maps, history of fly nuisance and other historical information, none of the countries performed a full breeding site prospection prior to identifying the potential first-line villages. In Burundi and Malawi, neither the presence of flies nor the distance to the river (both self-reported by village leaders) was associated with individual or village Ov16 prevalence. An onchocerciasis environmental suitability score was also not associated with village- level Ov16 prevalence.

Onchocerciasis is a focal disease that depends on the presence of a specific ecology – the presence of fast-flowing waters – and specific vector characteristics. We find onchocerciasis in communities near black fly vector breeding sites. Studies show a correlation between the proximity of villages to the water and the prevalence and intensity of onchocercal infection occurring in those villages, giving rise to the designations first-, second- and third-line villages. In mark-release-recapture studies, vectors have been found to travel up to 27 km; 15–20 km is reported for appetitive flights. Dispersal up to 60–100 km is seen for ovipositing females, who fly over rivers searching for suitable breeding sites. There is a marked difference in fly dispersal in the two main bioclimatic zones: parous (and therefore potentially infective) flies tend to remain close to the river in the savannah, while in the rainforest they disperse far from the river in search of a blood meal. There is an observed gradient in the prevalence of onchocerciasis related to distance from breeding sites in hyper-endemic areas. Biting rates decrease rapidly at increasing

distances from the river. First-line villages are those within 5 km of a breeding site, not just within 5 km of a river.

The procedure for identifying first-line villages is as follows: (i) stratify an area into zones based on ecological, entomological and demographic criteria, (ii) map the river sections in each zone, dividing the river into sections with high vector production, low vector production and no vector production areas, (iii) subdivide river sections into stretches about 50 km in length and (iv) record, within a selected river stretch, all villages closest to the breeding site and within 5 km of the river bank as first-line villages. When there are no human settlements close to the vector breeding site, the first-line village could be 10–20 km away from the breeding site. Importantly, vector breeding sites, and thus first-line villages, are not static, and changes that affect the location of vector breeding sites influence the designation of first- line communities: climate change (droughts, delayed rainy season), building dams and bridges, etc. There are potential new tools for identifying first-line villages, like a remote sensing model. Investing in such new tools will make it easier to find out where to do work to find breeding sites.

Discussion: The issue of transmission area was raised. The presenter explained that a buffer of 20 km around the breeding site would, in most cases, define a transmission area. It was then asked about how to conduct stage 1 sampling if there are no known breeding sites, no reports of black flies and people may not even know about black flies because they are never present. The presenter emphasized that the first step of OEM is exclusion mapping – conducting a desk review and collecting information in the community about black flies to identify areas where we cannot or do not have onchocerciasis, and to exclude those areas from any serological surveys. The river basins have always been the basis of onchocerciasis work. In exclusion mapping, you must look closely and identify areas of probable risk. You also need to look at nearby districts and breeding sites; administrative boundaries are not the best for determining where to look; you must consider the ecological/biological situation. If you have 1:50 000 maps, you can determine where there might be rapids, and, indeed, on some old maps the rapids are actually marked. If there are no black flies present in an area, then stage 1 OEM should not take place. But the programme needs to investigate adequately for the presence of black flies and take into consideration that some breeding sites could be seasonal, so more than one visit to confirm the presence or absence of black flies may be required.

5.2 Sampling methods for stage 1 mapping

Presentation: The goal of this presentation was to finalize the sampling method for stage 1 sampling. The purpose of stage 1 sampling is to identify areas where we are certain that MDA is required (meaning that the true prevalence of Ov16 is above the biologically meaningful threshold) using the most efficient approach possible. This means that, for stage 1 sampling, the programmes should look for signs of onchocerciasis in the most likely places, using a method that is quick and inexpensive. This presentation set out to answer three key questions needing resolution in order to finalize a stage 1 mapping strategy.

1. Should stage 1 decisions be based on the average district prevalence or the village level prevalence?

The presentation made clear that, for stage 1 sampling, it would be inappropriate for programmes to take the average of the first-line village results and compare it with the threshold. Instead, the decision to initiate MDA should be based on whether the maximum first-line village prevalence meets or exceeds the agreed upon threshold (if it does, then MDA is indicated). This is unchanged from the previous recommendations from the OTS. Programmes would need to review the data and decide whether district-wide or subdistrict MDA was indicated; additional testing may be required in sub-districts that are not felt to require treatment.

2. How many first- line village sites should be visited during stage 1 sampling?

Simulations comparing different numbers of first-line villages sampled (e.g. sampling 1 village versus 2, 3, … up to 10 villages) were presented, along with the proportion of times that these simulations

correctly classified the district as requiring MDA. These simulation results were used to find a balance between correctly classifying districts versus expending extra resources by going to more villages than necessary. Based on the evidence presented, the presenter suggested that increasing the number of first-line villages sampled in stage 1 from three villages to a minimum of five villages would improve the efficiency and accuracy of the overall mapping strategy by reducing the need for stage 2 OEM by 20% in districts that needed MDA in the modelling exercise. As the modeling exercise randomly selected villages, good entomological and local knowledge should improve the efficacy of stage 1. Investing more time in identifying first-line villages will reduce the need for additional mapping.

3. What is the optimal MDA decision-rule?

The presentation demonstrated how important the test performance characteristics are to the development of a statistical threshold for MDA decisions. Until a diagnostic tool with known sensitivity and specificity can be agreed upon, it is not possible to recommend a statistical threshold for MDA decisions. However, the presenter shared the logic sequence and calculations required for determining this threshold. It can quickly be adjusted once a test's performance is defined. The potential for false–positives, even with a 99% specific test, requires that the MDA decision-rule threshold be increased above the biological threshold (presently agreed to be 2% Ov16). One hypothetical example was provided: if the diagnostic test has a sensitivity of 80% and a specificity of 99%, then an appropriate decision-rule threshold, to be reasonably confident that the observed signal is not due to noise or sampling error, is 5% Ov16 prevalence. This means that if one or more of the five first-line villages sampled during stage 1 has an Ov16 prevalence $\geq$ 5%, then MDA is required and there is no need for further sampling. If all five villages have < 5% Ov16 prevalence (out of the 100 adults sampled per village), then no MDA decision can be made; instead, stage 2 random sampling is required. For stage 1 sampling, this decision-rule threshold would be designed to avoid falsely concluding that a truly non-endemic area (e.g. Ov16 prevalence of 0%) requires MDA.

The use of a 5% Ov16 threshold for making a treatment decision, as opposed to the 2% threshold initially proposed, demonstrates the need to identify a statistical threshold that is separate from the biological threshold. The present 2% biological threshold refers to the Ov16 prevalence below which we do not believe transmission can be sustained (e.g. the "break point"). The statistical threshold (e.g. $\geq$ 5% Ov16 prevalence in any one first-line village) is the decision rule we use to determine where MDA is needed; a decision rule is used to assess the biological threshold, and it accounts for the sampling design and imperfect performance of the diagnostic test.

The presentation was highly interactive, with decision points requiring participant input and agreement arising during the presentation; thus, much of the discussion occurred during the presentation. Meeting participants weighed and debated the assumptions and statistical constraints for each decision point. The first question (should stage 1 decisions be based on the average district prevalence or the village level prevalence?) arose because some NOEC interpreted the existing recommendation as possibly meaning that it would be appropriate to compare the *average* prevalence of the first-line villages to the threshold, rather than to compare the prevalence of each individual village with the threshold. The presenter highlighted that comparing the average prevalence across first- line villages would *not* be appropriate in the context of OEM because the disease is highly focal, particularly in low prevalence settings, and *all* transmission foci must be addressed. All present agreed that for stage 1, sampling first-line villages is appropriate. There was discussion around the fact that many studies that examined onchocerciasis prevalence in both first-line villages and randomly selected villages found that the prevalence in some of the randomly selected villages often exceeded the prevalence in the first-line villages. Nevertheless, there was agreement that where first-line villages are known, and where there is local, expert entomological knowledge, first-line villages can be accurately identified, and they will prove valuable for this stage 1 sampling.

In the hypothetical example provided in the presentation, it was determined that a *5% statistical threshold* would define the decision rule for starting MDA. This is the statistical threshold necessary to measure the *provisional biological threshold of 2%.* The 5% statistical threshold decision rule states

that if any of the five villages sampled has a prevalence above 5% then MDA should be initiated. Using a statistical threshold allows one to account for false–positives. If 100 adults in a village who are truly not infected are sampled, and the specificity of the test is 99%, then there is a 26% chance that two or more uninfected individuals will have positive test results, requiring MDA in an area with no transmission. If we use a statistical threshold of 5%, there is only a 0.3% chance that five of our 100 uninfected people will test positive. This dramatically reduces the likelihood of us identifying a village as being above the treatment threshold just by chance. If we then extend this approach to all five villages that we intend to sample, we find that if we set the statistical threshold at 5% then there is only a 2% chance that we will initiate MDA, when in fact the area is non-endemic.

Discussion: Consensus was reached that assessing 100 adults in each of at least five first-line villages (total 500) would improve our chances of correctly identifying areas where the prevalence of Ov16 positivity exceeds that biological threshold for transmission. A statistical threshold would need to be determined to adjust for test performance. The goal of stage 1 OEM is to quickly and efficiently identify districts or subdistricts of transmission and start MDA in those areas. If transmission is not identified in stage 1 OEM, then programmes should proceed to stage 2 OEM in order to exclude transmission in the evaluated area. However, programmes may decide to perform additional testing as part of stage 1 OEM, particularly if they believe that they should have found transmission, and if they can do so in a manner that is more cost effective that stage 2. This could include entomological surveys or the testing of additional first-line villages. However, stage 2 OEM would be required if MDA is not indicated by the additional testing.

Importantly, the 5% statistical threshold mentioned in the hypothetical example is dependent on the sensitivity and specificity of the test and will need to be adapted to account for the performance of the diagnostic test being used.

There was discussion around some of the assumptions that affect the determination of the treatment decision-rule threshold. One participant reported that models suggest that 2% is a very low prevalence for having sustained transmission. Others noted that the 2% threshold comes from examining untreated hypo-endemic villages. The OTS recommended it as a provisional threshold and has requested entomological studies be done to validate it or raise it. Also, from a programmatic perspective, there is a preference to incorrectly characterize places as needing treatment rather than incorrectly classifying them as not needing treatment, and then having to go back and map and/or treat later.

A concern was raised that there might be significant over-treatment, if for example only one first-line village exceeds the threshold and yet the whole area is treated. It was recalled that onchocerciasis is a focal disease. The presenter noted that countries can of course decide to map at a subdistrict level, but, in the stage 2 presentation, the presenter argued that stratification and treatment of certain areas should be allowed only if there is strong evidence that the disease is truly focal in that area. Others considered that the decision to map at a subdistrict level should be left to the countries and their NOECs, as the context is important. A district sandwiched between two districts under treatment may not need subdistrict mapping, and local ecological considerations could also be important. However, in evaluation areas in low - risk areas, there may not be enough known about the potential focality of transmission for subdistricts to be an option. The question was raised if one could divide an area in half to have a smaller geographical area to treat in the event of a focal distribution of positive results. Subdividing after stage 1 OEM would allow MDA to be started in the area with positive results. However, additional testing to complete the full OEM sampling requirements would be required in the area without positive results, in order to confirm that MDA is not warranted.

Finally, it was noted that the specificity of the test is driving this sampling strategy. Defining *true* specificity is challenging. A common set of samples that was used to compare any available test to a single standard would help make sure that thresholds and sample size were adjusted appropriately. It was noted that the specificity may be different in the field than in laboratories in the USA, highlighting that translation of this strategy to the real world is essential now and that we will need to do a few more tests to guide us in the real-world implementation of this OEM strategy.

5.3 Lessons from the field on stage 1 mapping

Presentation: This presentation summarized key lessons from Ghana and Nigeria during the process of conducting Sightsavers-supported OEM pilot projects in stage 1 first-line villages. Both countries found the "desk review" process useful; in Ghana it was essential to have an entomologist involved as early as possible and to cross- reference the initial first-line communities identified with NTD programme knowledge of black fly biting in those areas. In Nigeria, the Federal Ministry of Health was very involved in the desk review process, which was found to be useful, as it aided in the retrieval of archived data. It was noted that data on historical or recent breeding sites are frequently unavailable in ivermectin- naive areas, and in Nigeria identification of potential breeding sites was difficult due to rivers only flowing for a few months in each year, limiting the time during which entomological evaluations can be performed. Both countries noted that good community mobilization and clear messaging around the OEM surveys were essential to good participation in survey communities. Logistically, all required tasks could be completed by the field teams in the time allotted per community in each country. In Ghana, appointment of a dedicated logistician/quality control officer, WhatsApp for fast communication during surveys and daily team meetings were noted as key to the success of the survey.

Discussion: The committee asked whether Sightsavers could share the technical briefs for this work. The representative of Sightsavers replied that it was their intention to share the technical briefs once they were updated to incorporate any changes to OEM recommended by OTS. Details of supply chain issues were then discussed. There were challenges with the RDT import licenses and significant delays in delivery from the manufacturer. Programmes using the RDT will need to plan far ahead and allow significant lead- time for delivery of RDTs. A suggestion was made to investigate using the laboratory in Ouagadougou as a warehouse for RDT/ELISA supplies and have them ship to in-country WHO offices to reduce delays. A reasonable stock for multiple countries could be kept in such a store house and shipped on demand.

The selection of first-line villages is a critical part of the OEM strategy, and it can be challenging. For the pilot studies, previous knowledge about black fly presence together with onsite interactions with the community were the main criteria used for the selection. However, the investigators considered that collecting more detailed information about black flies would be helpful in identifying villages for testing. In these studies, they used only a yes/no question about the presence black flies' biting.

5.4 Sampling methods for stage 2 mapping

Presentation: The goal of this presentation was to finalize the sampling methodology for stage 2 sampling for OEM by defining the sampling strategy needed to determine whether a district (or subdistrict) should receive MDA for onchocerciasis. The purpose of the stage 2 sampling method is to find unknown foci of transmission or to be reasonably confident that the survey area is non-endemic and does not require MDA. Stage 2 sampling occurs only when stage 1 sampling in first-line villages does not find a signal of transmission (i.e. no village Ov16 prevalence exceeds the threshold for starting MDA). This presentation set out to answer six key questions needing resolution in order to finalize a stage 2 mapping strategy.

1. Should stage 2 decisions be based on the average district prevalence or the individual village- level prevalence?

Given the highly focal nature of onchocerciasis, and because the goal is elimination of transmission, the programme cannot ignore any villages with a clear Ov16 signal. Consequently, it is necessary to base stage 2 decisions on the village- level prevalence rather than the average district prevalence. This guidance differs from that of the first OTS meeting, but it is considered to be better aligned with the programmatic goals.

2. How should villages be selected for stage 2 sampling?

Two options for the selection of stage 2 sites were presented for the group to debate and ultimately select the preferred approach. The first option was to order villages geographically and then systematically select villages from the list. This method has the advantage of being easily operationalized and standardized across surveys, and it produces a spatially representative sample of villages. The second option is to allow countries to select villages based on their own risk assessment, prioritizing the selection of villages deemed to be at greatest risk of transmission (e.g. first-line villages not included in stage 1, proximity to rivers). The group decided that, based on the data presented from the OEM pilot studies, it is important to incorporate random sampling (by taking a systematic sample from an ordered list of villages) in stage 2. The most practical method would be to group villages into higher risk and lower risk villages (e.g. first-line, second-line and other).

3. How many village sites should be visited during stage 2 sampling?
Simulation results that compared different numbers of randomly chosen clusters were shown (ranging from five clusters to 50 clusters). While visiting more clusters will always increase the chances that a single village that exceeds the threshold will be found, the simulations demonstrated that after visiting 20 clusters, there appear to be diminishing returns in visiting more clusters. Consequently, the recommendation was to visit 20 clusters (i.e. villages).

4. How should sampling take place within selected villages?

Three options for selecting participants within villages were presented. Convenience sampling has the advantage of being simple and quick, and it provides an easy way to balance males and females; however, it cannot be standardized across sites and runs significant risk of leading to biased results. Systematic household sampling has the advantage of being the most rigorous approach that, when performed correctly, leads to an unbiased estimate of the true village prevalence; however, it may lead to an under sampling of men if they are more likely to be away from the household, and it requires village- level population information in advance, which is not always available at the national level. The Expanded Programme on Immunization (EPI) sampling method is well known by many health workers, simple to implement and reduces the risk of bias found in convenience sampling; however, as a quasi-random sampling method, it does not result in a truly unbiased estimate of the village prevalence. The group decided that EPI sampling provides the most feasible solution. It was noted that many programmes are already well-versed in this approach (also referred to as the "random walk" method), and it can enable programmes to achieve sex-balanced samples (sampling for a particular sex stops once you reach your target sample size). The group also discussed that it would be reasonable to apply this same sampling approach to the first-line villages in stage 1 to make it easier for programmes. However, purposive sampling should still be an option in stage 1 OEM.

5. What is the optimal MDA decision-rule?

For stage 2 sampling, it is important to optimize both the sensitivity of the strategy (i.e. the likelihood of correctly classifying an endemic district as requiring MDA) and the specificity (i.e. the likelihood of correctly classifying a *non-endemic* district as not requiring MDA). A hypothetical example was given.

> If 20 villages are included in stage 2 sampling, and test sensitivity was 80% and test specificity was 99%, it is not until at least two villages are observed with $\geq$ 5% prevalence that you can conclude that the district is endemic and that the positives observed are not due to chance (resulting from false–positives due to the imperfect specificity of the test). For a single village, it is not until the Ov16 prevalence is $\geq$ 10% that one can conclude with reasonable certainty that the signal is due to true Ov16 prevalence above the biological threshold and hence that MDA is indicated. Both of these thresholds assume that the specificity of the diagnostic test is 99% and the sensitivity is 80%. Taken together, the proposed decision-rule for stage 2 sampling is a two-tiered process: (i) if a single village (out of the 20 sampled) is $\geq$ 10%, then MDA is indicated; otherwise, (ii) if two or more villages are found to have a prevalence $\geq$ 5%, then

MDA is indicated. If neither condition is met, then the entire district can be considered non-endemic and does not require MDA. This is a strategy that can be tested in operational research and could be compared with alternatives proposed by other researchers. The actual thresholds and decision rule would need to be adjusted for performance of the test used in the strategy.

6. Can subdistrict decisions be made?

If the results from either stage 1 or stage 2 sampling suggest that only part of the district merits MDA, it is possible to stratify the district post-hoc to enable separate MDA decisions at the subdistrict level. The OTS recommends that this decision be reviewed with the NOEC or through consultation with WHO. As an example, if three villages are found to have an Ov16 prevalence above the MDA threshold out of the 20 sampled, and all three villages pertain to the same geographical area, then it is possible to stratify the district along administrative lines and apply the full decision-rule criteria to each stratum independently. Importantly, the full stage 2 survey will need to be completed in the stratum that has no villages above the MDA threshold if there is a desire to withhold MDA from this stratum (e.g. if there are 17 villages in the stratum that are all below the threshold, then an additional three villages will need to be sampled to complete the 20 stage 2 villages required). If the decision-rule is not exceeded (see question 5.4.5 above), then the stratum can be considered non-endemic. In this example, in the stratum with the three villages above the threshold, there is no need to complete the stage 2 sampling at the stratum level because it is already clear that MDA is indicated.

Discussion: As with the presentation on the approach to stage 1 sampling, this was a highly interactive presentation, with much discussion during the presentation so that consensus on certain questions was reached before the end of the presentation. For other points, debate continued beyond the formal presentation. The challenge in this effort was to find an approach to each step of the sampling that was statistically robust (and therefore predictive of the true onchocerciasis situation), but also practical and feasible. The goal of OEM is to maximize the number of endemic districts identified for treatment and minimize the number of non-endemic districts identified for treatment. As previously stated, test performance drives much of the discussion and the determination of the threshold.

Regarding the first question, as for stage 1, the group readily concluded that the stage 2 decision to treat should be based on individual village prevalence results, rather than the average prevalence across all randomly selected villages; again, this was because the disease is highly focal and, in the context of elimination, all transmission foci must be addressed. This was supported by the idea that if there is one village with a significant prevalence, you cannot take the average prevalence of all the villages and then decide not to treat. A single village should not be ignored.

For the second question, the ideal approach would be geospatial sampling, with villages selected according to the scale of spatial correlation. Such a strategy would require the GPS coordinates for every village in advance; however, because this is challenging, it was agreed that spatial representation could be approximated by ordering villages geographically (e.g. list villages starting from the northwestern part of the district down to those in the southeastern part of the district) and then selecting systematically from the list. A second approach was to allow countries to select villages using their own criteria and incorporate local knowledge, to favour those areas with the greatest risk of transmission. A third approach is to sample as many clusters as is feasible using systematic random sampling. This generated lively discussion. There was a lot of support for relying on local knowledge to select higher risk villages.

The group returned to the discussion of whether entomological and local expertise really could identify first-line or other high-risk villages, and the fact that, in many studies, some randomly selected villages have higher prevalence than first-line villages. The group agreed that there are many settings where indeed, local knowledge and entomological expertise can identify villages likely to harbour transmission, even if the prevalence in these villages is not always higher than some randomly selected villages. However, the group ultimately settled on systematic random sampling. If the experts' selection of the best first-line villages to sample in stage 1 failed to produce a signal indicating that MDA should

be initiated, there is no reason to believe that this approach would be more successful if another five villages were selected, and so stage 2 sampling should use the systematic approach. However, there was agreement that we need to be very explicit about how to order villages for the sampling.

A suggested way forward was to group villages by risk (if known), listing first-line villages, followed by second-line villages, followed by all others (or just first-line and all others) and to create a systematic random sample from the grouped list. There was also some discussion around the idea of doing the purposeful (stage 1) and systematic random sampling (stage 2) in a single stage. There might be cost and time implications either way; it may be less expensive to start with just the first-line villages, but if you do not detect a signal then you have to go back into the field. There was some concern that people would not apply themselves to do a rigorous job of stage 1 sampling if they know there is a stage 2, but it was agreed that the approach needs to be implementable by countries without strong entomology capacity. There was also discussion about whether or not the approach might be different in savannah areas versus forest areas. Forest areas have been acknowledged for decades as being areas where identifying all breeding sites is challenging.

The decision to select 20 clusters seemed clear from the simulations presented. It was suggested that if the geographical area was large, then the number of clusters could be increased; however, the decision rule for determining whether MDA is needed would need to be able to account for a variable number of clusters. This is possible and has been done in other surveys (e.g. transmission assessment surveys for LF).

Regarding how to sample individuals within the selected sites, there was general agreement that EPI random walk sampling offers a good balance of rigour and convenience. There were concerns that convenience sampling could be biased to finding those people at lowest risk for exposure, so some effort was indicated to avoid this. However, precise prevalence estimates are not required, and thus EPI random walk sampling should be sufficient.

To identify the optimal MDA decision rule (that is, the statistical threshold above which MDA should be initiated), an in-depth statistical comparison of different thresholds was presented. However, unlike in stage 1 where we wanted to maximize specificity, in stage 2 we want to maximize both specificity and sensitivity (that is, we want to avoid treating non-endemic districts and to ensure that we do not incorrectly identify endemic districts as being non-endemic). It was necessary to strike a balance. If the statistical threshold for triggering MDA is too low, then we risk treating when not necessary, but if the statistical threshold is too high, then we risk poor performance of our approach in a low (but > 2% biological prevalence) setting. Again, the calculations are contingent upon the assumption that 2% prevalence represents the biological threshold above which transmission can be sustained. The decision rule suggested, as any decision rule, cannot address all situations, which created discomfort among many programme leaders and experts. For example, in the hypothetical example, if one village had 9% prevalence and six villages had 4%, they would not have met the decision rule for starting MDA. It would not be possible to devise rules for every possible scenario. Instead, national programmes should review the results and consult their NOECs and/or WHO to arrive at a decision on the way forward in these circumstances.

The question was raised about what to do if the sensitivity of the mapping tool is < 80%. The decision rules can be adjusted for this. Once cost estimates for mapping are available, the cost of using less sensitive tests can be generated. Generating a table of what OEM would look like using tools with 60% and 70% sensitivity would be helpful. One could increase the number of people per village, or the number of villages, or we could lower the threshold, but we would have to "tolerate" the fact that we would be more likely to initiate MDA when the district is not in fact endemic.

Some raised concern about the random sampling approach proposed for the stage 2 OEM sampling and whether one should map more first-line villages if transmission was not identified in stage 1. The concern was that there might not be much chance of finding onchocerciasis in randomly sampled villages if the first-line villages are all negative. This generated much discussion. Data indicate that

randomly sampled villages frequently reveal some villages where the prevalence is actually higher than previously identified first-line villages. Others defended the ability of entomologists and programmes to correctly identify first-line villages. Some noted that countries' designation of a village as first-line may have been based on old data. Reassessment of breeding site activity may have identified changes in the status of black fly activity and allowed reclassification of villages. But the point of the systematic random sampling is that it will follow *after* one's best effort to identify the highest risk first-line villages. As the best effort was made to identify first-line villages in stage 1, a different approach is needed for the stage 2 sampling.

5.5 Experience with stage 2 mapping

Presentation: This presentation summarized key lessons from Ghana and Nigeria in conducting OEM pilot projects supported by Sightsavers in stage 2 randomly selected villages. The performance of RDT and SD ELISA was compared, and the prevalence of Ov16 antibodies as detected by RDT and ELISA was compared and contrasted between first-line and randomly selected villages in each cluster. In Ghana, the SD or AP ELISA signal in first-line villages was > 2% in all villages, although first-line villages did not always have the highest prevalence. In the one evaluation unit for which SD ELISA results were available in Nigeria, again, the SD ELISA signal in first -line villages was > 2%. For RDT, highest prevalence was again found in non-first-line villages.

It was noted that, in Ghana, the mapped areas were in densely riverine, forest areas, whereas in Nigeria, mapping was conducted in dry savannah areas. Maps were presented showing the challenges of identifying first-line villages in the forest areas, given the high number and close proximity of rivers. Even the savannah area in Nigeria presented challenges because of seasonal streams. In Ghana, the threshold for starting MDA was reached after the RDT survey of first-line villages, and further surveys were done for research purposes only, not for decision- making. In Nigeria, RDT prevalence was < 2% in all first-line villages in both LGAs, but SD ELISA analysis of DBS in first-line villages indicated the threshold had been reached in the one LGA for which ELISA results were available.

Discussion: Country representatives were asked whether there were issues with the random sampling. The greatest challenge was in enumerating/labelling all the households in the village. It was pointed out that if the threshold for starting MDA was met, then although stage 2 mapping was not needed, it would still be important for the programme to determine the location of the other breeding sites in the area. The importance of identifying breeding site locations should be added to any guidance or training materials. A comment was made that in selecting first-line communities for mapping, we should continue to advise countries to start with villages near meso- and hyper-endemic areas, rather than near a river where little is known about the risk of transmission, to increase the chance of finding a real signal. Although this has been the recommendation of the OTS in the past, it appears that the message has been lost. We are still in the process of learning about novel methods for excluding areas that do not need mapping and identifying breeding sites more easily. It would be reasonable to hold off on mapping areas about which we know very little while more piloting of mapping procedures takes place. Overall, there was consensus that this mapping approach appears to work well, even though the onchocerciasis community is not perfect at identifying first-line villages.

5.6 Additional lessons from pilot surveys of stage 1 and 2 mapping

Presentation: This presentation summarized cross-cutting issues and lessons learnt across both stages of OEM pilot surveys supported by Sightsavers. It was noted that procurement for RDTs, including import permits and customs waivers, was a time-consuming process with lead- times in excess of 12 weeks in each country. The unavailability of positive control and daily QC panels for RDTs was noted as an issue in 2/4 of the evaluation units surveyed. This has been resolved for future Sightsavers pilot OEM surveys after a discussion with PATH. The lack of sensitivity of the RDT when compared with the ELISA is an issue in both Ghana and Nigeria for the SD ELISA, and in Ghana where the AP ELISA was also run and had similar levels of increased sensitivity when compared with RDT. This creates

confusion on how these results should be interpreted and acted upon. It was further noted that some RDT- positive samples were negative by SD ELISA, which further complicates data interpretation and onward decision making.

The experience with developing a standard set of training materials, technical briefs and checklists was outlined. Next steps for these materials include their review and edit to include final OTS protocol recommendations for OEM, and then their review by external partners and eventual use as potential resources for future OEM surveys. The use of the EPSEN Collect electronic data collection tool and metabase processing data server were reviewed. Costing was touched upon briefly, but ELISA analysis is ongoing and costs are still accruing in some categories, so detailed breakdowns will be available as soon as this is complete. Finally, there was a summary of how the Sightsavers experience to date with OEM pilots has informed the approach in the final pilot country, Mozambique.

Discussion: There was considerable discussion around the issues of the supply chain, which was also touched on in a previous presentation about the OEM experience. There was a very long lead- time for ordering RDT or ELISA kits. Production does not begin until the tests have been paid for and all importation documentation has been provided. As the shelf- life of the RDT is 12–18 months, in-country stocks will need to be managed carefully. It was reiterated that one solution would be to centralize the purchase and storage of these tests. There could be a forecasting system to allow the central stores to know how many tests countries anticipate ordering.

The committee was happy to see that Sightsavers had generated an OEM checklist/critical pathway that should be useful to others trying to implement this activity.

Cost details were not shared, but a significant proportion of the costs related to laboratory supplies: US$ 19 000 for the SD ELISA kits plus US$ 15 000 for shipping them. An OEM budget calculator has been developed to help programmes with planning/costing. Every effort should be made to minimize mapping where it is not needed; a good exclusion mapping process is essential. Eliminating the need to ship the SD ELISA kits with cold chain would also significantly reduce the cost for those areas that need testing in addition to RDT testing.

5.7 Onchocerciasis mapping in areas co-endemic for LF

Presentation: This presentation summarized the OTS discussions concerning OEM in areas co-endemic for LF and the data presented to the committee to date. The question is whether onchocerciasis elimination programmes can make use of LF prevalence evaluations for OEM, specifically the LF transmission assessment survey, or TAS. There are three possible scenarios in which onchocerciasis prevalence testing might be combined with the TAS for OEM:

1. TAS is conducted and the onchocerciasis prevalence is found to be at or above the threshold for starting MDA for onchocerciasis. In this scenario, MDA with ivermectin alone would continue after albendazole is stopped for the treatment of LF.
2. TAS is conducted and the onchocerciasis prevalence is found to be below the threshold for starting MDA for onchocerciasis. In this scenario, MDA with ivermectin and albendazole will stop once TAS confirms LF transmission is below the LF threshold.
3. The Global Programme to Eliminate LF has provided ivermectin for $\geq$ 10 years, so the Onchocerciasis Elimination Program can proceed directly to an onchocerciasis pre-stop evaluation or to an onchocerciasis stop-MDA evaluation.

The bottom line for all three scenarios is that any onchocerciasis prevalence signal requires either MDA or demonstration of interruption of transmission.

Successful integration of onchocerciasis prevalence into the TAS depends on the age group to be evaluated. The TAS surveys children aged 6–7 years, while the OEM guidelines suggest testing adults aged $\geq$ 20 years old in ivermectin-naive areas. However, areas in which LF and onchocerciasis are co-endemic are not ivermectin-naive areas. This raises multiple questions:

- *Are adults the most informative age group for OEM in these areas?*
- *Would altering the TAS age range beyond 6–7 years old be worth the information versus the resources?*
- *If children are the most informative group, would school-based sampling vs. community-based sampling be acceptable?*
- *What threshold should be used to trigger MDA?*

To help answer some of these questions, operational research in Burundi and Malawi in 2018 compared Ov16 seroprevalence obtained using village-based sampling of adults aged ≥ 20 years versus school-based sampling of children aged about 10–14 years. In all districts in Malawi, the correlation between village and school prevalence was moderate at best. The number of students living in their matched village ranged from 36% to 89% by district, indicating there was some catchment from other villages. We observed that numerous randomly selected villages had onchocerciasis prevalence higher than preselected first-line villages based on REMO mapping, suggesting that the programmes may not have a robust method of identifying first-line villages. Examining results in children compared with adults, in some villages we got the expected result of adult prevalence exceeding that in children; however, in multiple villages onchocerciasis was detected in children but not in adults and a number of villages detected higher seroprevalence among children than among adults. These patterns were observed in both countries and were counterintuitive. Therefore, there was an unclear relationship between village and school prevalence data in Burundi and Malawi. These results could be reflective of actual transmission patterns or differences in catchment area, in which case they need to be explained, or reflect error caused by imprecise data due to small sample size and test sensitivity, or problems with the internal or external validity of the study.

The group was then asked to discuss the key questions posed during the presentation.

Discussion: The discussion began by examining the relatively higher prevalence among schoolchildren than adults. There have been reports of biting rates being higher in schools, so the findings may be correct. In Uganda, the biting rate in classrooms (using human landing collections) was three to seven times greater than near the rivers. Others commented that the most reasonable interpretation of the data would indeed be that the children are experiencing greater exposure/infection, either at school or elsewhere. On the one hand, there was concern about using children as a proxy for adults, given the lack of correlation between children and adults (or school and home location). On the other hand, if you see a signal in children then you are done and should proceed to MDA. It was pointed out that the data that were presented are from 10–14-year-old children and that children in a TAS are aged 6–7 years, so it is not appropriate to extrapolate from these data to what might be found in a TAS. Many thought that we should stick with testing adults in this setting, as this is the approach that is being recommended for mapping generally. Current WHO recommendations suggest adding Ov16 testing to LF TAS when feasible. This could still be done. In situations where there were positive results above the threshold for start MDA in adults, you would be able justify continuation of MDA for onchocerciasis and just stop the albendazole for LF. In situations where the results were negative, additional data would be needed (e.g. OEM with exclusion mapping followed, if indicated, by testing in adults or full stop MDA assessment for onchocerciasis). There were insufficient data to make a formal recommendation about testing in adults.

5.8 Recommendations on onchocerciasis elimination mapping

1. Exclusion mapping (or identification of unmapped areas where the environmental is unfavourable for the presence of black flies) is important and should be undertaken before proceeding to stage 1 mapping.

2. Mapping should begin in high- risk areas (e.g. those near hyper- and meso- endemic districts, or where previous surveys found the presence of onchocerciasis). National programmes may want to delay OEM in lower risk areas until more data from pilot studies become available.
3. Stage 1: Purposeful sampling of first-line villages
    a. Select five first-line (or high- risk) villages.
    b. Draw a convenience sample of 100 adults.
    c. Test using eluted DBS on RDT if programmes have no prior experience with ELISA; save the leftover DBS. If ELISA is used, good documentation of QA/QC procedures and recording of results is essential.
    d. If Ov16 prevalence in one or more villages exceeds the statistical threshold, then initiate MDA.
    e. If Ov16 prevalence in all sites is less than the statistical threshold, then proceed to stage 2 as merited by context.
4. Stage 2: Random sampling of villages
    a. Recommendations for stage 2 sampling are for operational research purposes only at this time.
    b. Group villages by risk (e.g. first-line villages not included in the stage 1 OEM, followed by second-line villages, then the remaining villages).
    c. Systematically select 20 villages from the grouped list of villages.
    d. Sample 50 adults per village using EPI (random walk) sampling, with an effort made to achieve an equal balance of men and women.
    e. Test using eluted DBS on RDT or ELISA as described in 3c above.
    f. If Ov16 prevalence is ≥ 5% in **two or more** villages **or** ≥ 10% in one or more villages, then initiate MDA; this threshold should be adjusted based on the performance of the test used.
    g. If the above criteria are not met, and there are no additional concerns, then no MDA is indicated.
5. If infection appears focal (positive villages are clustered in one area), subdistrict decisions can be made, and the survey area can be stratified post-hoc; however, in each stratum that is not targeted for treatment the total sample size should be completed by sampling additional villages to reach the full complement of 20 and the criteria for initiating MDA must be reapplied to that strata using data from all 20 villages. The NOEC or WHO should be consulted to review this information
6. Additional research is needed to:
    a. Assess the spatial correlation of Ov16 and O150 PCR in different ecological settings (e.g. savannah versus forest) to determine its applicability in hypo- endemic areas.
    b. Determine the appropriate Ov16 biological threshold (prevalence) that is consistent with the potential for ongoing transmission; entomological indicators should be part of this assessment.
7. There is not a recommendation to combine OEM with TAS surveys at this time; however, TAS surveys should still be used to evaluate transmission and, if transmission is found, ivermectin MDA should continue.

6. Maximizing the available diagnostic tools

The group returned to a discussion of what can be done to maximize use of the available diagnostic tools.

There was agreement that we need to revisit the target product profile (TPP for onchocerciasis diagnostics at the various stages of the programme (e.g. mapping, stopping MDA, PTS). It was agreed that we do not want the programme to be driven by a diagnostic: we want the programme needs to drive the development of the diagnostic. Once the TPP is established for each setting, it can be determined

what needs to be done to develop a diagnostic that fits the TPP (e.g. make a small change to an existing diagnostic or develop a completely novel diagnostic).

At the same time, it was agreed that it is important to be pragmatic about the immediate need to provide advice to allow programmes to move forward. The evidence for the use of Ov16 in each setting is significant, including published evidence from the Region of the Americas. Essentially, the question is about the format of the tool and its operationalization in new ways, at large scale, to help programmes move forward.

The group provisionally agreed to recommend using the RDT on DBS for mapping. Countries with experience with ELISA can continue to use ELISA for OEM provided appropriate QA/QC is in place. A simple next step would be to test the RDT on DBS using recently collected field samples and in field laboratories, to assess the sensitivity and specificity of the test. It is important to be very clear about the target use for the test, the reasonable expectations for the test and the acceptable performance criteria. Moving forward, a common strategy for evaluating performance that everybody agrees on needs to be prepared and implemented so that final recommendations can be made.

It is very important, despite the challenges, to use a standard sample set and provide it to laboratories in different settings both in the USA and in endemic countries to evaluate the reproducibility in the field of the boosting effect of using RDT on DBS. This common sample set would standardize comparisons across settings and laboratories. There was strong support for such a panel, including snip PCR positives and defined negatives from non-endemic or post-endemic areas, as a means of defining sensitivity and specificity; it will be important to include samples from non-endemic settings that have challenged other tests (unless it can be demonstrated that the unexpected results were due to something other than a potential problem with the ELISA).

There are also a lot of field samples awaiting analysis in order to make conclusions about starting MDA. It may be necessary to proceed with analysis of these samples while the details of the tests are resolved. Although it would be ideal to run these in a reference laboratory, there are too many samples to process in any one place. Performing analysis in regional or national laboratories that have the appropriate training and support would help develop capacity while providing data that could allow programmes to make decisions. But national programmes must be supported to move forward while the diagnostic issues are being resolved.

There was consensus that we should move ahead with the two evaluation ELISAs that were reviewed at this meeting (AP and SD ELISAs), but also that the OEPA AP ELISA should be included in the standardized analyses. However, in terms of operationalizing an assay across all the endemic countries, there was a strong belief that the assay needs to be lot controlled; there needs to be a single lot of antigen that would be made commercially with lot control, and the number of independent reagents (independent variables in the ELISA process) needs to be minimized. The RDT and OEPA ELISA could be compared for sensitivity. It would also be helpful to determine if the poor sensitivity of the RDT in the field is related to the test or to some field performance issue.

Data can be reviewed as complete analyses become available by calling for interim teleconferences rather than waiting for the next meeting of the OTS. The terms of reference of this committee allow for this to happen, and committee members and other key informants will be called to meet via teleconference as needed.

7. Updates

7.1 Stop MDA threshold

Presentation: There are concerns that the present serological threshold for making stop MDA decisions (Ov16 < 0.1%) may be lower than is biologically necessary. Based on empirical evidence from countries that that have stopped MDA, when there were no infected flies or simply no flies to be collected, the Ov16 prevalence in children aged 5–9 years ranged from 0.02% to 1.3% (by AP or OEPA ELISA). Results from the NTD Modelling Consortium suggest that elimination can be achieved with 95% certainty with a serological threshold of 2% among children aged 5–9 years, or with 99% certainty if the threshold is reduced to about ~1%. According to these same models, one can be even more certain that elimination has been achieved if the age group is expanded to include children aged 5–14 years. Together, these data suggest that the present threshold is too stringent and that it may be appropriate to raise the serological threshold for stopping decisions to 1%. Given the current state of onchocerciasis diagnostics, the only tool that appears well-suited for making these stopping decisions is an ELISA, as data from two different studies suggest that the Ov16 RDT in its present format is insufficiently sensitive for detecting low positive signals in the target age group of young children. Operational research is needed to validate the appropriate serological threshold for stopping MDA. This can be done by increasing the number of sites where both serology and entomology data are collected, as well as conducting repeat cross-sectional surveys in areas that have stopped treatment and where entomology indicates that transmission has stopped but a serology signal remains.

Discussion: The threshold should be slightly different depending on the operational sensitivity of the assay. This is an important observation. It may be possible to develop different thresholds, one specific to the assay being used, though this would need to take into account both the sensitivity and the specificity of the test. Implementing countries would need guidance in interpreting their data with respect to thresholds when different assays were used (and the relevant sample size). This issue also related to the previous discussion of the need to accurately determine test performance characteristics outside of reference laboratories. But experience and testing of thresholds will yield additional information. Of note, all the current antibody-based diagnostics will be challenged by efforts to accelerate progress towards the interruption of transmission (e.g. vector control or treatment twice yearly with ivermectin). In these situations, it may be necessary to wait for the serology to catch up with reality: transmission reduction may happen faster than the reduced antibody prevalence in children, and using the older group of children (10–14 years) may not be ideal, as serological markers in that group will take that many years longer to reflect any actual reduction in transmission.

7.2 New diagnostics

Presentation: Having taken a multi-"omic" approach to the stage- specific expression of RNA and proteins in most of the vertebrate and invertebrate stages of the *O. volvulus* parasite, potential biomarkers for fertile adult female parasites were identified. Based on the exclusive expression of particular genes in adult females (i.e. little to no expression in other stages), two genes were identified (OvOC8995 and OvOC12838) that had the additional benefit of having no homology to human or other helminths and being secreted. Using multiple immunogenic peptides from each antigen, mono-specific affinity purified rabbit antibodies were generated and used to configure antigen capture immunoassays for both proteins. These assays first showed limits of detection of 3 ng/mL for OvOC8995 and 0.5 ng/mL for OvOC12838. When tested on large panels of mf+ *O. volvulus*-infected patients and compared with a variety of control sera, both assays were able to detect antigen in the overwhelming majority of infected patients (combined sensitivity of ~89% at 99% specificity). Moreover, following definitive treatment in a small subset of patients followed longitudinally, the levels of both antigens dropped dramatically; some disappeared altogether. Monoclonal antibodies to both antigens have been made and are being readied to replace the rabbit antibodies that are likely to add to the sensitivity of these two assays. Validation cohorts are being readied prior to making the assays available for programmatic use.

Discussion: The Committee agreed that the tests looked promising and encouraged the continued development of the test.

7.3 Milestones for elimination: developing the road map to 2030

Presentation: The 2020 goals for onchocerciasis include: elimination of transmission in the Americas by 2015 (now updated to 2022); elimination of transmission in selected countries in Africa by 2020 (APOC set 2025 goal for 80% of countries; individual countries are now setting their own goals); and elimination of transmission in Yemen by 2015.

Looking towards 2030, Sustainable Development Goal (SDG) 3.3 states: "By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases". This has been operationalized by WHO as a 90% reduction in the burden of neglected tropical diseases. SDG milestones for onchocerciasis should be country-level, meaningful steps towards the achievement of the elimination of onchocerciasis and should both include process and impact milestones. They should be trackable, with target dates when possible. This may require adaptation of data collection systems, or subnational modelling to make projections. Stakeholder input has been sought on these milestones, with variable success.

Potential process milestones include:

- onchocerciasis elimination mapping completed in all countries;
- 100% geographical coverage achieved in all countries;
- minimally effective coverage of 65% of the total population reached in all countries;
- access to quality- assured laboratory testing available in all countries;
- capacity to implement entomological surveys available in all countries;
- strategy for MDA in low prevalence areas co-endemic for loaiasis developed;
- strategy for MDA in low prevalence areas co-endemic for loaiasis implemented in all affected countries;
- WHO guideline for post-elimination surveillance developed; and
- onchocerciasis included in country UHC packages of care in all endemic countries.

Potential impact milestones include:

- total population no longer needing MDA;
- number of countries that have stopped MDA in at least one transmission area;
- number of countries that have completed PTS in at least one transmission area;
- number of countries verified by WHO as having eliminated onchocerciasis;
- number of countries in which 25% or more/50% or more/75% or more/or 100% of the population no longer need MDA for onchocerciasis (*meaning PTS has been completed)*.

Discussion: The discussion touched on a variety of different points. There was general support for the impact milestones, though some disagreement about whether measuring the percentage of the population where MDA has been stopped or where PTS has been completed would be more appropriate. The MDA-has-been-stopped option would be achieved earlier and demonstrate progress faster than the PTS-has-been-completed option. There was a proposal to add the definition of transmission zones in all countries as a process milestone; to add progress with exclusion mapping as a milestone; and to add identification of breeding sites as a milestone. There was a suggestion to make a request that national master plans include a certain set of components. There was also a note that both sides, WHO and countries, should be held responsible for achieving these milestones. There was consensus that countries should not be overburdened by being asked to collect a lot of data that they are not already collecting. Modelling using M&E data can help countries assess their progress and forecast resource needs. WHO will bring these suggestions forward and combine them with other stakeholder input as it becomes available.

7.4 Pre-stop MDA assessments

A brief comment was added to note the current WHO-recommended approach for conducting pre-stop MDA assessments. The pre-stop MDA assessment provides a means of rapidly assessing, at relatively low cost and effort, whether an area that has been under MDA warrants the larger expenditure of time, effort and resources to complete a full stop MDA assessment. This interim recommendation provides some guidance for countries uncertain about how to determine when to implement a full stop MDA assessment.

The current method proposed for a pre-stop MDA survey is to select three to five first-line villages that are associated with known, productive breeding sites, select a sample of 100 children aged 5–9 years and test using whichever test the programme prefers. If two or fewer children are positive, then move forward with full stop MDA assessment.

Annex 1. Meeting agenda

Day I	Item	Name
	I. Opening session	
09:00–09:15	Welcome	WHO
09:15–09:30	Introductions	ALL
09:30–09:45	Review meeting objectives	Cantey
09:45–10:00	Highlights of second OTS meeting	Unnasch
10:00–10:30	Review of serological tests	Won/Golden
10:30–11:00	**Coffee break**	
	II. Country presentations	
11:00–11:30	Mali: stop MDA	Sow
11:30–12:00	United Republic of Tanzania: nearing stop MDA	Upendo
12:00–12:30	Togo: nearing stop MDA	Togo/Bronzan
12:30–13:30	**Lunch break**	
13:30–14:00	Malawi: mapping and nearing stop MDA studies	Laston
14:00–14:30	Nigeria: mapping plus a question of thresholds	Igbe
14:30–15:00	Ghana: mapping	Marfo
15:00–15:30	Kenya: mapping	Kenya
15:30–16:00	**Coffee break**	
16:0 –16:30	Burundi: mapping	Bucumi
16:30–17:00	Ethiopia: a question of thresholds	Secretariat
17:00–17:30	Uganda experience with laboratory	Oguttu
17:30–18:00	Discussion	
18:00	**END OF DAY 1**	

Meeting agenda (cont'd)

Day II	Item	Name
	III: ELISA standardization	
09:00–09:30	Summary of ELISA comparisons	Won
09:30–10:00	Quality assurance, work flow, and cost of ELISAs	Won/Golden
10:00–10:30	Discussion of ELISA to support for QA	ALL
10:30–11:00	**Coffee break**	
11:00–11:30	Wrap-up of discussion of ELISA	ALL
	IV: RDT	
11:30–11:45	DBS RDT comparison	Won
11:45–12:15	Efforts to improve RDT (DBS or dual antigen strip)	Kenya/ Nutman/Won
12:15–12:30	Summary of ELISA versus RDT comparison	Golden
12:30–13:30	**Lunch break**	
13:30–14:30	Discussion about role of RDT in programme activities	ALL
	V: Onchocerciasis elimination mapping	
14:30–15:00	Sampling methods for stage 1	Gass
15:00–15:30	Lessons from the field for stage 1	Hamill/ Countries
15:30–16:00	**Coffee break**	
16:00–16:30	Identification of first- line villages	Boakye/Gass
16:30–17:00	Discussion of first- stage mapping	ALL
17:00–17:30	Mapping in areas co-endemic for LF	Roy
17:30–18:00	Discussion	ALL
18:00	**End of Day II**	

Group reservation at 19:30

Day III	Item	Name
	V: Onchocerciasis elimination mapping	
09:00–09:30	Sampling methods for stage 2 mapping, number of clusters and sample size	Gass
09:30–10:00	Experience with stage 2 mapping	Hamill/Gass
10:00–10:30	Lessons from pilot surveys of stage 2 mapping	Hamill/Countries
10:30–11:00	**Coffee break**	
11:00–11:30	Discussion of stage 2 mapping	ALL
11:30–12:00	Timeline for finalization of OEM Programme Guide	Cantey
	VI: Updates	
12:00–12:30	Update on the stop MDA threshold	Gass
12:30–13:30	**Lunch break**	
13:30–14:00	Updates on new diagnostics for onchocerciasis: US NIH	Nutman
14:00–14:30	Discussion	ALL
14:30–15:00	SDG 3.3 and the need for milestones	Cantey
15:00–15:30	Discussion of potential milestones for onchocerciasis elimination	ALL
15:30–16:00	**Coffee break**	
16:00–16:30	Discussion of potential milestones for onchocerciasis elimination	ALL
16:30–17:00	Other business/closing	
17:00	**END**	

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