Report on the fourth meeting of the WHO Onchocerciasis Technical Advisory Subgroup

Virtual meeting, 28–29 October 2020
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<tr>
<td>APOC</td>
<td>African Programme for Onchocerciasis Control</td>
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<tr>
<td>DBS</td>
<td>dried blood spot</td>
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<td>DTAG</td>
<td>Diagnostic Technical Advisory Group</td>
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<td>FTS</td>
<td>filariasis test strip</td>
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<td>LF</td>
<td>lymphatic filariasis</td>
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<tr>
<td>MDA</td>
<td>mass drug administration</td>
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<tr>
<td>NTD</td>
<td>neglected tropical disease</td>
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<td>OCP</td>
<td>Onchocerciasis Control Programme</td>
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<td>OEM</td>
<td>onchocerciasis elimination mapping</td>
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<td>OTS</td>
<td>Onchocerciasis Technical Advisory Subgroup</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PES</td>
<td>post-elimination surveillance</td>
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<td>PTS</td>
<td>post-treatment surveillance</td>
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<td>qPCR</td>
<td>quantitative real-time PCR</td>
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<td>RDT</td>
<td>rapid diagnostic test</td>
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<td>TAS</td>
<td>transmission assessment survey</td>
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<td>TPP</td>
<td>target product profile</td>
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<td>World Health Organization</td>
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Executive summary

The fourth meeting of the Onchocerciasis Technical Advisory Subgroup (OTS) of the World Health Organization (WHO) was held virtually on 28–29 October 2020. The meeting reviewed some important issues and approved recommendations on (i) the Ov16 enzyme-linked immunosorbent assay (ELISA) for decisions on stopping mass drug administration (MDA) and mapping elimination of onchocerciasis; (ii) integrated evaluations for onchocerciasis and lymphatic filariasis (LF); (iii) country feedback on onchocerciasis elimination; and (iv) updates on the black fly diagnostic initiative, dried blood spot (DBS) rapid diagnostic test (RDT) and on the draft entomological manual for onchocerciasis elimination programmes.

1. Ov16 ELISA for stop-MDA and onchocerciasis elimination mapping

OTS examined comparative data on the Ov16 ELISA from a variety of settings in African countries. These data have been disappointing, although work is still ongoing. No decision was made on the Ov16 ELISA platform, but this should not stop programmes from moving forward with their activities. It was recognized that despite having no recommended ELISA platform, MDA was successfully stopped in over 7.5 million people with the current diagnostic supported by O-150 polymerase chain reaction (PCR).

Recommendations

- Comparison of the evaluations should continue, focusing on performance characteristics, reproducibility of results and logistical feasibility.
- Countries should continue with the ELISA they are already using.
- Entomological evaluations will be key while work continues on the development of ideal serological methods;
- Quality assurance is essential to ensure that programmes can make decisions based on accurate data.

For mapping using Ov16 serology, OTS was unable to recommend a format for onchocerciasis elimination mapping (OEM). However, there was evidence which showed that performing RDT with DBS rather than using fresh blood in the field increases the sensitivity of the Ov16 RDT. Further issues discussed included determination of a lack of transmission, and programmes were advised to conduct exclusion mapping before proceeding to select first- or second-line villages. OTS also deliberated on first- and second-stage sampling methods in OEM, providing the number of villages and adults to be sampled in each stage including the relevant decision-making thresholds.

Recommendations

- Evidence has demonstrated that using DBS increases the sensitivity of the Ov16 RDT, so it is recommended to use the RDT on DBS and to save DBS for future analysis by ELISA.
- Mapping should start in high-risk areas, i.e. in areas with known transmission or with known black fly biting.
- Mapping should begin with exclusion mapping, i.e. in areas where black flies cannot breed or where the environment will not support black flies.

2. Integration of onchocerciasis and LF evaluations

The current WHO recommendations for programmes require that they align the timing of LF and onchocerciasis impact assessments. Where feasible, transmission assessment surveys (TAS) and epidemiological or entomological impact assessments can be conducted together to maximize use of resources and coordinate decisions on stopping treatment. The current challenge is stopping MDA in LF-onchocerciasis co-endemic
areas. OTS discussed ways in which it might be possible to combine LF with onchocerciasis evaluations in pre-TAS or TAS 1, based on the various case scenarios presented.

In areas “known to have onchocerciasis”, it was suggested that if LF is ready for pre-TAS, then it would be possible to proceed with an LF/onchocerciasis co-evaluation. There might be one LF sentinel site and one spot-check site, whereby everyone aged above 5 years would be tested, equating to some 300 people at each site. There would be 3−5 onchocerciasis first-line villages, where only 5−9-year-olds would be tested.

In areas “without known onchocerciasis” cases, evaluation would, in step one, determine if a habitat is suitable for black flies. If not, onchocerciasis mapping would not be carried out. The second step would consist of integrated pre-TAS/OEM, whereby one LF sentinel site with everyone older than 5 years would be tested, or five first-line villages with everyone older than 5 years would be tested. There may or may not be LF spot-checks, depending on whether one of the first-line villages could also serve as the LF spot-check site.

OTS discussed a range of issues related to TAS and OEM that focused on selection of villages, pre-stop MDA, stop-MDA and village versus school settings. From the additional country co-evaluation experiences, OTS was able to derive some recommendations that would help programmes to undertake integration.

**Recommendations**

- In situations where black flies test negative after five rounds of MDA, that another black fly PCR should be repeated before taking a decision to stop MDA.
- Operational research is needed to establish whether five rounds of MDA are enough to eliminate transmission.
- If PCR testing is positive, treatment should be continued.
- Testing of adults should be kept as an option.
- OTS should make the co-evaluation of TAS1/OEM more specific in order to guide programmes in proper decision-making.
- There should be more field studies in the co-evaluation strategy to generate data from a variety of geographical settings, in order to support proper decision-making regarding stop-MDA.

**3. Country feedback on onchocerciasis elimination**

Feedback on onchocerciasis elimination efforts in African countries was received from the Democratic Republic of the Congo and Uganda. The Ugandan programme raised an issue relating to post-elimination surveillance (PES) not having been performed in most of the foci in the country in which post-treatment surveillance (PTS) has been completed. The Democratic Republic of the Congo had two issues with diagnostic tools, namely mapping in hypo-endemic areas and conducting assessments in ivermectin-naive areas.

**Recommendations**

- OTS should address PES issues in subsequent meetings in order to present proper procedures on PES to guide programmes and for incorporation in the revised WHO guidelines for verification of onchocerciasis elimination.
- Countries should integrate PES into the ongoing national health surveillance system for it to be sustainable.

**4. Onchocerciasis molecular black fly diagnostic initiative**

Global Health in collaboration with the WHO Department of Control of Neglected Tropical Diseases has launched an initiative with the goal of developing a diagnostic tool suitable for routine use that would be reliable and affordable. It engaged three major laboratories, which compared the sensitivity and specificities of tests
on pools of 100 black flies. The results from the three laboratories were promising. A range of issues was discussed by OTS, including endorsement/guidance to programmes on this test and its sensitivity, specificity and costs involved in the diagnostic initiative.

**Recommendations**

- The NIH O150 should be the primary test and the OvND5 should be used as an independent confirmatory assay, in parallel, rather than in multiplex.
- The quantitative real-time PCR (qPCR) method is recommended because it is simpler and faster to use and has higher sensitivity and specificity.
- All reagents should be supplied to laboratories in pre-prepared freeze-dried 96 well plates.
- The silica-based DNA extraction and purification method was efficient for isolating DNA from the black fly pools and should therefore be standardized in forthcoming standard operating procedures.

5. **DBS on RDT protocol update**

OTS discussed the results of data presented from various field studies. There was variable good performance of DBS RDT in different settings, and, in all settings, DBS RDT performed better than RDT. The studies from the three laboratories concluded that the performance of DBS on RDT had comparable sensitivity to the SD ELISA, while specificity was higher than the SD ELISA.

**Recommendations**

- To take DBS into the field, ELISA should not be used because the results are inconsistent.
- Programmes should use RDTs run from DBS because they are easy to perform.
- There should be independent confirmatory tests to validate ELISA.

6. **Update on the draft entomological manual for programme managers**

OTS discussed the presented draft entomological manual, focusing on updating the chapters with innovative vector control strategies, publication, training in the use of this manual including its roll out, and translation into French and Portuguese.

**Recommendations**

- The current review of the entomological manual, involving specialists in the various fields, should take into consideration the application of the document in the field, and include areas such as handling of PTS in appropriate sections.
- WHO should endeavour to make the entomological manual available for field use as soon as possible in order to support national programmes in their elimination efforts.
- WHO should plan to translate the manual into French and Portuguese as soon as it becomes available to prevent Francophone and Lusophone countries from lagging behind.
1. Introduction

Due to the ongoing COVID-19 pandemic, the fourth meeting of the World Health Organization’s Onchocerciasis Technical Advisory Subgroup (OTS) was held virtually on 28–29 October 2020. Dr Dieudonné Sankara, Team Lead, Eradication and Elimination, WHO Department of Control of Neglected Tropical Diseases, welcomed the participants to the meeting. He thanked everyone for joining, and for committing their time given members’ busy schedules. He pointed out that this was an important meeting, geared towards the prevention of preventable blindness and disabilities due to onchocerciasis.

Welcoming remarks from WHO and statements in honour of the late Dr Ricardo Thompson were delivered before the discussion.

The agenda is reproduced as Annex 1 and the participants are listed in Annex 2.

1.1. Opening remarks

Dr Mwelecele Ntuli Malecela, Director, WHO Department of Control of Neglected Tropical Diseases, began by recognizing the achievements of the onchocerciasis community over the past 10 years. She reflected on progress in the elimination of onchocerciasis, as published in the Weekly Epidemiological Record. In 2019, 156 million people received treatment for onchocerciasis, achieving geographical coverage of 86.9%. More than 1.8 million people now live in areas where post-treatment surveillance (PTS) has been completed and mass drug administration (MDA) is no longer required. This has been a great achievement. Importantly, she added, and looking ahead with the road map for neglected tropical diseases (NTDs) for 2021–2030, WHO has set ambitious but achievable goals. WHO aims to verify 12 countries as being free of onchocerciasis and 34 countries as having stopped MDA in at least one transmission focus, and this despite the impact of COVID-19 on programmes.

WHO is moving forward to address diagnostics as a priority, and the meeting would be updated on progress in developing a black fly polymerase chain reaction (PCR) method to support programme needs, Dr Malecela said, while ensuring that programmes have the tools they need to measure progress. The Diagnostic Technical Advisory Group (DTAG) has made significant progress in developing initial target product profiles (TPPs) for several diseases, including the onchocerciasis subgroup. She said that an update on the status of the onchocerciasis TPPs would be given to the meeting later on the first day.

Entomological capacity is an important requirement for onchocerciasis elimination. WHO has led the development of a new programme managers’ manual that presents the lessons learnt from a decade of experience from the Onchocerciasis Control Programme (OCP) and the African Programme for Onchocerciasis Control (APOC). The updated manual provides updates since 2002 on new tools and methodologies that have been proven effective and will be a welcome resource for programmes, Dr Malecela said. This manual too, it was noted, would receive a status update during the course of the meeting, and a comparative study on black fly PCR methodology would also be presented.

WHO has been looking at the road map and reviewing integration of NTD activities in the evaluation of onchocerciasis so as to maximize use of scarce resources. WHO has also long recommended evaluation of onchocerciasis wherever a transmission assessment survey (TAS) for lymphatic filariasis (LF) is performed. Programmes need guidance, Dr Malecela said, on how best to approach this at different stages, particularly when mapping for hypo-endemic onchocerciasis. Guidance on how to proceed with integrated assessments is certainly needed, and the NTD department looked forward to reviewing the outcomes of the OTS’s discussions.

Dr Malecela reiterated that many countries will be embarking on LF TAS activities in the coming months in areas that may need onchocerciasis mapping. She had hoped that more progress would have been made by now in diagnostic comparison studies and in the Stage 2 mapping protocol, but noted that the COVID-19 pandemic had affected
much of the necessary scientific work. Completion of this work was to be looked at when it will again be safe to resume activities, she noted.

In conclusion, she commended the support provided by Merck to countries that are resuming MDA despite resources being stretched and scarce in these countries. These additional resources were extremely welcome and would greatly benefit countries resuming activities. Members were thanked for committing their time to the meeting. Dr Malecela ended her remarks by acknowledging their important input as WHO embarks on the road map for 2021−2030, which requires addressing and realigning of strategies in order to accelerate onchocerciasis elimination.

Dr Paul Cantey was made chair of the OTS.

1.2. Highlights from the third meeting

OTS reviewed the highlights from its previous (third) meeting (Geneva, 26−28 February 2019).

- **Review of comparisons of Ov16 ELISA for evaluating stopping decisions** The results of comparisons of Ov16 ELISA from a variety of settings in African countries have been disappointing. However, work is continuing to compare formats to determine performance characteristics, reproducibility and logistical feasibility. In this regard, because OTS was unable to make any decisions on this, programmes could not be prevented from moving forward with their activities where there is no data on which OTS can advise. MDA has been successfully stopped in more than 7.5 million people using the current diagnostic, along with black fly PCR. Therefore it is important that programmes focus on making sure that they have good entomological evaluation, coupled with good quality assurance for proper decision-making.

  - The following decisions/recommendations were made:
    1. Continue the comparison with the evaluation focusing on performance characteristics, reproducibility of results and logistical feasibility.
    2. Countries can continue with the ELISA they are already using.

- **Ov16 serology for mapping**

OTS was not able to recommend a format for onchocerciasis elimination mapping (OEM) using Ov16 serology. Evidence showed that the RDT performed from DBS improves the sensitivity of the RDT, compared with the use of whole blood, while avoiding some of the specificity concerns seen with the Ov16 SD ELISA. This, for now, is the recommendation for programmes undertaking onchocerciasis mapping. The recommendations from the first and second OTS meetings had been that mapping should always proceed from the high-risk areas.

In onchocerciasis elimination mapping (OEM), there is always a challenge to determine how much data is enough to determine lack of transmission. To address this challenge, a transmission zone can be divided to balance the known and the unknown. It is always obvious that the traditional breeding sites are closer to the river and are at higher risk for ongoing transmission. The highest risk of transmission is near breeding sites, but, in circumstances where breeding sites are unknown or cannot be located, an alternative strategy is required. The group discussed that random sampling or sampling along rivers could be used as the best proxy for breeding sites.

  - The following decisions/recommendations were made:
    1. Evidence demonstrated that using DBS increased sensitivity of the Ov16 RDT, so the recommendation was to use the RDT on DBS and to save DBS for future analysis by ELISA, if necessary.
    2. A positive signal by RDT is meaningful, but, because of the sensitivity concerns, negative results are not informative.

    2. Mapping should start in high-risk areas – areas with known transmission or areas with known black fly biting.
**Onchocerciasis elimination mapping**

- **Stage 1.** In first-line OEM, you select five first-line villages and sample 100 adults, collect DBS and then do follow up ELISA if needed with ELISA RDT. The threshold to start MDA is 2% but this is still theoretical, based on modelling, and has to be adjusted according to the diagnostic test used and the findings from ongoing research to determine the empirical serological threshold that equates with no ongoing transmission.

- **Stage 2.** If the results from all five villages show no evidence of infection, there is a need to rethink. There may be no transmission; however, it is also possible that knowledge of the location of breeding sites may be imperfect. In this type of scenario, and given imperfect knowledge of breeding sites, you randomly sample 20 of the remaining villages, then test 50 adults in each of the 20 villages. If one village is > 10% or 2 villages > 5% Ov16, MDA is needed. This threshold should be adjusted based on the diagnostic test used and any revisions to the threshold. Entomology should be incorporated in the context of operational research to validate the thresholds. The summarized OEM algorithm emphases were based on exclusion mapping and good identification of first-line villages that, when properly done, could reduce the need for Stage 2 OEM. Stage 2 OEM is not ready for programme use but is ready for operational use.

  - The following decisions/recommendations were made:

  1. Start with exclusion mapping based on physical and ecological features of the area – areas where black flies cannot breed or where the environment will not support black flies.

  2. Map areas at highest risk first, using the existing WHO recommended strategy

  3. There is not yet a consensus on how to perform Stage 2 OEM if no signal is observed in Stage 1. The recommended strategy is outlined in the report of the third OTS meeting.

- **Other recommendations**

  - Studies are needed to determine whether a higher stopping threshold can be used [in progress].

  - Once the diagnostic test for stopping is finalized, the sample size will need to be recalculated based on the sensitivity and specificity of the test [in progress].

  - Work is needed to develop entomological capacity [in progress].

  - Diagnostics that truly fit programme needs should be developed [in progress].

  - Definition of 2021–2030 road map goals [completed – ratified at the Seventy-third World Health Assembly (9–14 November 2020)].

### 1.3. WHO 2021–2030 road map goals: interruption of transmission

The committee reviewed the WHO road map for 2021–2030 that is due for publication in December 2020. It is anticipated that this may be finalized during the World Health Assembly that will be held virtually in November 2020. For onchocerciasis, more than one milestone has been proposed because verification by WHO is very stringent given the need to interrupt transmission, stop MDA and conduct PTS in order to obtain verification. Stopping MDA in at least one focus is imperative for programmes. Importantly, however, almost every country could have stopped MDA in at least one focus by 2030. In summary, WHO aims to verify 12 countries as free of onchocerciasis and 34 countries as having stopped MDA in at least one transmission focus by 2030.
2. Evaluation

The meeting heard presentations on two main topics:

- integration of onchocerciasis and LF evaluations; and
- a co-evaluation of onchocerciasis and LF in the United Republic of Tanzania.

These presentations were followed by a discussion of the co-evaluation findings.

2.1. Integration of onchocerciasis and LF evaluations

Paul Cantey began the discussion by stating that integrated surveys are needed to avoid stopping ivermectin treatment in areas that pass TAS before it being discovered later that the area in question is in fact endemic for onchocerciasis and requires additional treatment with ivermectin. The current WHO recommendations to programmes are that they align the timing of LF and onchocerciasis impact assessments. Where feasible, TAS and epidemiological or entomological impact assessments can be conducted together to maximize use of resources and coordinate decisions on stopping treatment. In order for this to be done, modified sampling strategies may be required. Programmes still face the challenging issue of stopping MDA in areas where LF and onchocerciasis are co-endemic. It was noted that there is a lot of work ongoing in the field of onchocerciasis.

2.1.1. Assessments

- LF assessments
  - Pre-TAS
    - Assess 1 sentinel village and 1 spot-check village in a high-risk area
    - Sample 300 community members ≥ 5 and test with the filariasis test strip (FTS)
    - If the villages pass, proceed to TAS 1
  - TAS
    - Assess 30 clusters
    - Sample 1500–1700 children in school, ages 6–7 and test with FTS

- Onchocerciasis assessments
  - No defined pre-stop MDA assessment, but the following has been proposed:
    - 3–5 first-line villages
    - 100 children, ages 5–9 by RDT from DBS or ELISA
  - Stop MDA (defined by WHO guidelines)
    - Variable number of villages, often 30 with a mix of first-line and other villages
    - Test 3000 children ages 5–9 by ELISA
    - Entomological assessment

The presentation then considered ways in which it might be possible to combine LF with onchocerciasis evaluations in pre-TAS or TAS 1.

In areas known to have onchocerciasis, it was suggested that if LF is ready for pre-TAS, then it would be possible to proceed with an LF/onchocerciasis co-evaluation.

There might be one LF sentinel site and one spot-check site, where there would be testing for everyone above 5 years, equating to some 300 people at each site.

There would be 3–5 onchocerciasis first-line villages, where only 5–9-year-olds would be tested.

Furthermore, if the LF spot-check villages were situated in an onchocerciasis first-line village, this might serve two purposes; in the case of the LF evaluation being ready for TAS and onchocerciasis needing more data, it would be possible to proceed to integrated TAS 1. This would mean having some 30 villages/schools, stratified by status/association with first-line villages, then testing some 3000 children aged 5–9 years (Fig. 1).

Per these considerations, it was noted that assessments cannot be fully integrated because the age groups are different and different sample sizes are needed; although it would be for individual programmes to decide whether there might be cost savings to be derived from conducting both assessments at the same time. Therefore, sampling may need to be adjusted to ensure equal representation of ages. This also further assumes
that school sampling is equivalent to village sampling. There may be concerns about having too many villages or schools from areas that are not hyper- or meso-endemic; this would need to be supplemented by entomological results.

The results of these assessments, it was stated, might be interpreted in two ways:

- Integrated TAS with 5−9-year-olds and use this as the basis for an LF decision rule
  - This may constitute a more conservative interpretation and be more likely to fail TAS 1
- Adjust sampling to ensure equal representation of all age groups.

In areas without known onchocerciasis cases, evaluation would, in step one, determine if a habitat is suitable for black flies. If not, onchocerciasis mapping would not be carried out. The second step would consist of integrated pre-TAS/OEM, wherein there would be one LF sentinel site with everyone older than 5 years and five first-line villages with everyone older than 5 years. There may or may not be LF spot-checks, depending on whether one of the first-line villages could also serve as the LF spot-check site.

Following this evaluation, if prevalence exceeds the OEM threshold for Stage 1 mapping, mass administration of ivermectin might be continued regardless of TAS results. Where the prevalence falls below the threshold, it would then be apposite to proceed to integrated TAS 1/OEM.

An integrated TAS 1/OEM would include 30 villages/schools, stratified by status as first-line villages, and 3000 children, aged 5−9 years. If prevalence were found to be above the OEM threshold, ivermectin MDA would continue. If prevalence were found to be below the threshold for stopping MDA (0.1%), no onchocerciasis MDA would be needed (Fig. 2).

In cases where prevalence was found to exceed the 0.1% stopping threshold but below the starting threshold, the procedure would then go along one of the following paths:

- PCR on black flies; or
- Stage 2, mapping (adults only); or
- wait for recommendation on stage 2 threshold.

Other considerations in this context might include the fact that Stage 2 OEM has not been finalized, albeit with a likelihood that integrated TAS/OEM would exceed requirements for OEM. This should therefore be acceptable but would come with a higher cost requirement.
Fig. 2. Co-evaluation of LF in unknown onchocerciasis areas, integrated pre-TAS/OEM

2.1.2. Case scenarios

For integrated TAS 2/3-onchocerciasis assessments, it was suggested that if MDA for LF has been stopped but there is known onchocerciasis in the area, the onchocerciasis pre-stop assessment would be totally independent of the LF decision and could be conducted at any time, using the standard protocol. In circumstances where onchocerciasis is at pre-stop MDA, ~3 onchocerciasis first-line villages will be selected and children aged 5–9 years examined. If the results indicate that onchocerciasis does not need more data, then LF TAS 2/3 can proceed, but, if the result shows the opposite, then integrated TAS 2/3 will be required. In integrated TAS 2/3, 30 villages/schools should be selected and stratified by first-line village or not for LF, while for onchocerciasis, sample 3000 children in the 5–9 years age group and examine them. Once the results indicate prevalence above the OEM threshold, ivermectin MDA should be initiated. However, if prevalence is below the OEM threshold, other considerations should follow. If the prevalence is below the stop-MDA threshold, the area is declared to have no onchocerciasis and MDA is not needed. But if the prevalence is above stop-MDA and below OEM thresholds, black fly PCR should be conducted, or adults examined.

2.1.3. Discussion

The discussion was based on the selection of villages. Hyper-and meso-endemic areas were seen to be problematic to field teams, and it remains possible that these areas can be missed, leading to missing some positive cases. In the presentation it was stated that there must be a guarantee of first-line villages when selecting villages for TAS 1. It was noted, however, that village selection is often carried out using the sample survey builder. What, therefore, ought to be done if there were not at least one first-line village among the 30 villages selected by the sample survey builder?
In response to this, it was noted that when one lists the villages from the random selection by order of distance from river or likely first-line village, there will be a mix. Therefore, one may decide that it is necessary to pick 2–3 villages specifically for onchocerciasis. Furthermore, when villages are selected, it must be taken into account that there may be some where the survey cannot be implemented, leading to the selection of five additional villages. Some of these ought to be onchocerciasis first-line villages. If not, still other villages would need to be selected for onchocerciasis.

*The meeting registered the need to include a note about these five additional villages in protocol descriptions.*

On the evaluation related only to OEM or to pre-stop and stop-MDA evaluations, in terms of the time interval between starting treatment and conducting pre-TAS, there was concern about how this would be linked with onchocerciasis evaluations or OEM monitoring and evaluation, or decisions to pre-stop or stop MDA evaluations?

It was observed, however, that the evaluation was based on an onchocerciasis perspective. The relevant pathway would depend on onchocerciasis needs, be they OEM, monitoring and evaluation, or stopping surveys. The proposal outlined above is for pre-TAS, or in the case of TAS happening soon. For long-term planning, there is a chance to secure more coordination.
On the question of whether the 30 villages from the sample size builder were for the TAS or the pre-TAS, it was clarified that this was related to TAS, and that pre-TAS consisted of two village sites, mostly sentinel and spot-check sites.

There was concern whether there is a lower limit in the number of schoolchildren to be sampled during TAS. Two propositions were advanced:

1. In situations where you cannot get the 1500–1700 sample, test everyone; and
2. Include additional schools to get your sample size required.

In the school versus village settings, it was observed that schoolchildren can come from many villages or also from the same village, and this could compromise the results. It is not recommended that everyone be tested if a sufficiently large sample cannot be secured. In the absence of a correct sample size, the survey sample builder can be used to select alternate clusters.

Regarding the choice of the first-line villages, it was clarified that once all first line-villages have been checked during the pre-TAS, it is acceptable to have a more random selection subsequently to check the entire area during the integrated LF/onchocerciasis TAS. Also related was the question of GPS coordinates for selected schools/villages that were not always known before the TAS is carried out, which could lead to difficulties in prioritizing by distance to river or breeding site.

The response to this question noted that, in practice, communities/villages are grouped and aligned according to their proximity and that the sample survey builder is used to sample the requisite 30 villages plus five as back up. While it is true that coordinates are not always available, those working in a district know very well where villages are and always help to group villages based on their proximity.

2.2. Co-evaluation of LF and onchocerciasis in the United Republic of Tanzania

The United Republic of Tanzania has a population of 53 million people, and LF and onchocerciasis are co-endemic. Mapping for both diseases was conducted in 1990–2012. The population at risk for LF is 53 million while that for onchocerciasis it is 6.3 million. There have been 18–20 annual rounds of MDA in the endemic areas. A study on co-evaluation of LF and onchocerciasis using different platforms was conducted using four platforms. Dr Upendo Mwingira presented this report on a co-evaluation of LF and onchocerciasis in the United Republic of Tanzania.

Four specific examples of such co-evaluations were cited:

- **Example 1:** Onchocerciasis monitoring in LF pre-TAS, also TAS setting where four communities were selected
  - 1 LF sentinel site, three front-line villages
  - Sampled 100 children in each village, ages 5–9

- **Example 2:** Integrated school-based TAS
  - Sampled 3000 children for onchocerciasis, ages 5–9 years
  - Samples analysed for LF on FTS

- **Example 3:** Onchocerciasis monitoring in school-based TAS 2
  - Sampled 3000 children between grades 1 and 4
  - Children in grades 1 and 2 were tested on FTS, Ov16 and DBS for ELISA was collected
  - Samples from children in grades 3 and 4 were sampled for DBS on ELISA only

- **Example 4:** Onchocerciasis monitoring in LF community based TAS 2

**Example 1: Results of OV monitoring in pre-TAS**

In this example, the results from the four villages were all negative for FTS.

**Example 2: Results of OV monitoring in TAS1**

In the 35 schools where 2551 children were tested, two FTS were positive but the results for the DBS have not yet been analysed.

**Example 3: Results of OV monitoring in LF TAS2**

In this setting, more than 15 rounds of MDA have been conducted (passed LF TAS1 and due for LF TAS2). The monitoring was nested in a TAS survey where the sample survey builder was used to select 30 schools of classes 1, 2, 3 and 4 in the age range of 6–9 years. Classes 1 and 2 were targeted for FTS, Ov16 and DBS for ELISA, while
Example 4: Ov16 monitoring in TAS2-community set up

This was a multi-country LF-TAS study involving American Samoa, Haiti and the United Republic of Tanzania. The objective was to test the sensitivity of TAS for detecting evidence of recent transmission in an evaluation unit. The area selected (Muheza) had passed TAS1 in 2004 and had 13 immunochromatographic test (ICT)-positive children with a cut-off of 20. The neighbouring district (Lushoto) passed TAS in 2004 and there was a 0-ICT positive. The study was to verify whether there is still sustainable transmission in the area. Some 69 hamlets were selected from the area. For LF TAS, 1540 children aged 6−9 years were selected, for onchocerciasis 1275 children (aged 8−9 years) were selected and community members (1692) aged > 10 years were sampled. Adults were included for research purposes. The diagnostic tests used were antigen versus antibody. The results, which were plotted on a map, showed that for Ov16 there were some positive spots while for LF the positive cases were scattered throughout the district.

Summarizing the lessons learnt from the co-evaluation, it was stated that co-evaluations require a high degree of coordination between LF and onchocerciasis programmes; that RDTs were useful for securing immediate results; and that co-evaluations of the type described led to meaningful results that were used to make programmatic decisions.

There were, however, challenges that need consideration. These included limited access to RDTs, meaning that central procurement and funding are needed; limited laboratory capacity for running ELISA, which requires continued training in protocol updates; and the slow process of running Ov16 ELISA in the laboratory. The test kit is expensive, yet consensus has not yet been reached by OTS on which ELISA programmes should use. For instance, the Tanzanian programme had to have training from the United States Centers for Disease Control and Prevention (Atlanta, GA, USA) for its laboratory and field teams.

2.2.1. Discussion

There was concern regarding a map showing the FTS and Ov16 positives; this was thought to be appropriate when put together.

This was followed by a question about the third example given. Might not the 9-year-olds cause a bias in the evaluation, given that they would have received MDA? If their onchocerciasis results were negative, might this have been because they had had LF MDA?

Regarding the 9-year-olds, Dr Mwingira responded that they might have received MDA for 2 years, but that it depended on whether one was discussing LF or onchocerciasis results. If discussing LF results, a positive test would likely mean positive for current infection, and one would not expect antigenaemia to change after only 2 years of treatment. However, if one was talking about the onchocerciasis results, these are antibody tests, and one would not expect infection to be cleared after only 2 years of treatment.

Other subsequent concerns centred on how one can easily miss onchocerciasis communities when looking at clustering. When carrying out integrated evaluation, it was noted that there is a need to ensure that one is in an area at risk of onchocerciasis. If not, there may be a risk of making the wrong decision (e.g. stopping treatment).

It was pointed out that this type of situation was the reason Stage 2 sampling in OEM is carried out.

A further comment regarding concerns about the focality of onchocerciasis noted that the inclusion of the pre-stop MDA survey (i.e. five purposeful first-line sites), as well as the 30 clusters for LF, would increase the chances of a survey identifying ongoing onchocerciasis transmission. It ought to be borne in mind, it was noted, that LF TAS sampling is not completely random; rather, it is a geographically stratified sample.

In this situation, Dr Mwingira responded, it was known that part of the district was endemic for onchocerciasis. The random sampling across the district identified the hyperendemic area and some of the hypo-endemic areas. This emphasizes the importance of context as well as thresholds. In this case, the programme was able to expand its understanding of where onchocerciasis might be located, because a lot of the villages on the eastern side were not known to be hyper- or meso-endemic areas.
It was agreed that consideration of environmental factors was vital to ensure the selection of villages where one would be most likely to find onchocerciasis, if present.

Differences in ecology and breeding preferences also meant that great care was needed in selecting villages, a further respondent added. Breeding sites require special attention to ensure that villages near those sites are selected.

Critical concern was raised as to whether, at the start of the LF programme, onchocerciasis covered the entire district (Muheza) in the study area. It was noted that this was a community-directed treatment with ivermectin area before LF programme commencement and that RDT-positive cases were from hilly meso-endemic areas.

There was concern also about the cost of evaluation in terms of logistics and human resources. Logistically, it was stated, there were cost savings, because vehicles, in the situation presented, were all going to the same place. There was, however, a need to increase the number of laboratory technicians. It remained difficult to estimate the cost to the programme because additional support was received which covered some of the acquired costs. The programme itself was mainly responsible for the Level of Effectiveness (LOE) but, logistically, the approach described saved time and was preferred by community members.

2.2.2. Recommendations

Following the evaluation, the results were shared with the Tanzania Onchocerciasis Expert Advisory Committee (TOEAC), and two key recommendations were made:

1. Twice a year treatment with ivermectin was initiated in Mahenge and Tanga foci.
2. Finalization of assessment in Morogoro focus to know the status.

2.2.3. Co-evaluation discussion

Integrated pre-TAS/OEM (i)

The evaluation session concluded with a wide-ranging discussion of many issues relating to co-evaluation that merited further consideration.

Concern was raised about the need for a sample size of 3000, whether the goal was to reach that 0.1%, or whether the figure could be something above that.

In this case it was noted that the figure of 3000 was based on the assumption that one is trying to measure the 0.1% threshold; as the threshold increases, the sample size is reduced.

A scenario was also considered where there have been six rounds of ivermectin and albendazole MDA, and whether or not the 0.1% threshold would be considered in such a situation. It was suggested that in order to consider OEM/starting, a 2% threshold is considered and, in order to make a decision to stop MDA, one considers the 0.1% threshold. After six rounds of MDA, however, one is in a situation in between. It was further observed that it would be reasonable to expect a higher threshold in a situation where there is a lower sample size, bearing in mind that some programmes may not be able to do this on this scale.

It was proposed that in such a situation, it would be necessary to combine the sample (n=1700) of the 6−7-year-olds with sampling of 5−9-year-olds. It would, therefore, be difficult to go much lower than 3000 and still get meaningful data from 5−9-year-olds. If you sample 2000 children, which is the sample size for the OEM protocol, there would only be 3000 children outside of the 6−7 years age group. That would affect all the thresholds. At present, it is impossible to determine if 5−6 years of treatment in a hypo-endemic area can eliminate transmission.

The issue of school versus community-based sampling was raised. In this case it was proposed that there would be need for operational research to address the question but that in the absence of such work, would it still be possible to move forward? There were many propositions related to this subject:

One concern was the possibility of a concentration of children from many villages. In some settings, schools enrol children in the same village. However, school-based testing remains the most convenient way to sample. There was concern about a possible lower limit in the number of schoolchildren to be sampled during TAS. Two propositions were advanced:

1. In such situations where you cannot get the 1500−1700 sample, test everyone; and
2. Include additional schools to get the required sample size. In the school versus village
settings, it was observed that schoolchildren can come from many villages or also from the same village, and this could compromise the results. In appraising the co-evaluation strategy, it was suggested that the TAS1/OEM should be more specific in order to guide programmes. The negatives emanating from the evaluation results should be considered carefully, or when they are below the OEM threshold.

The meeting was informed that a study by the Task Force for Global Health compared school versus community sampling. In this the results were not definitive, even if there were instances where prevalence in schools exceeded that found in adults in communities.

It was noted that this depends on prevalence being above the OEM threshold. However, it is known also that the threshold has a number of factors, such as sensitivity and specificity of the test. Therefore, it would be prudent to provide guidance with tentative figures. For example, if one is using a test with x sensitivity and y specificity, a minimal sample will by z. Even the figure of 3000 children proposed here is based on a certain sensitivity and specificity.

To which it was noted the figure of 3000 children is based on the current protocols for stopping MDA, and that there have not been any adjustments to consider the performance of the test. There is a need to specify the threshold based on the test, in order to inform countries about the thresholds to use depending on which tests they use. This guidance, it was stated, needs to be disseminated.

**Integrated pre-TAS/OEM (ii)**

The group then considered a situation in which pre-TAS/OEM first-line village mapping has been completed. In such a situation, there may be three possible outcomes:

- If prevalence is above the stopping threshold, but below the starting threshold, the situation is unclear. Black flies can be tested, one can go back and test results, or one waits until there is more operational research to allow an answer to the question.

There was also discussion of hypo-endemic villages with negative black flies that could possibly turn to positive. Suggestions were made, and there was a belief that if after five MDA rounds the black flies test negative, it would be better to take another black fly test before taking a decision to stop MDA. This argument raised an important question whether five years of MDA is enough to eliminate transmission. If there has been good coverage during 5–6 years of treatment, transmission could theoretically have been suppressed and one might not see any positive flies. However, it was noted that negative tests need to be treated carefully. Negative flies may in fact represent transmission suppression after 5 years of LF MDA. Another respondent stated that one might not expect 5 years of treatment to be enough to stop onchocerciasis transmission, while another added that positive flies after 5 years of LF MDA might be indicative of a need to continue treatment. Negative flies are not, it was stated, an indication that treatment may be stopped. The APOC manuals used to state that very low hypo-endemic settings may suppress, rather than interrupt, transmission in 5 years.

Further suggestions were that, where treatment is given for years in a particular area and where black fly tests show no signal, it would be wise to initiate a further round of black fly testing. There is a need to know also how many black flies to test. After 5 years of treatment, if the flies are negative, it would be advisable to test again, and rigorous entomology evaluations would be a necessity. There was no consensus, and the question was deferred until there is adequate data to allow OTS respond to it. Other suggestions were to wait or conduct operational research.

On the positives, participants felt this could be an indicator of transmission and continuing ivermectin MDA would be appropriate. However, for the negatives, additional data would always be required. In this, members wondered whether it would be appropriate to test adults or conduct skin-snip PCR tests on the positive samples.
However, it was noted that skin-snip PCR is not highly recommended and may not be sensitive enough to be included in the discussion here.

A further consideration was that some of the areas discussed hitherto were unknown, and therefore may have been hypo-endemic. Onchocerciasis treatment would not therefore have been in place before the introduction of the LF programme. If so, it was noted, one cannot talk about first-line villages because there is a need to discuss breeding sites first.

Another participant noted that when conducting evaluation for onchocerciasis, following the rigorous procedures approved by WHO is paramount. For one transmission zone, a minimum of 6000 flies are required to be caught for the purposes of PCR testing. In the same transmission zone, a minimum of 3000 children aged < 10 years also need to be evaluated to measure the impact of ivermectin treatment. When an area is treated for up to 15 years, especially in hyper- and meso-endemic areas, you tend to get impressive results from the stop MDA assessment. In Nigeria, it was shown that many years of treatment in hyper- and meso-endemic areas, alongside good coverage, tends to reduce infection in hypo-endemic areas.

Given the challenges with interpreting negative flies, in hypo-endemic settings where the serological results are inconclusive, would it not be advisable to wait (without treatment) and retest during the next TAS? If transmission has not been interrupted, would one expect to see a signal in the children 2 years later? This proposal was accepted; however, one of the challenges observed in this suggestion is that one might have to resume MDA.

The issue of geographical and transmission zones which might include more than one district was also deliberated on, noting that the focus hitherto had been mainly on the district level.

The point was then made that if there is a need to conduct black fly PCR testing, there are steps that need to happen, programmatically, before one can go out and catch flies (if the area where onchocerciasis status is unknown). The debate could perhaps be resolved by advocating for entomological evaluation, i.e. before testing, there is a need to know where breeding sites might be, the best time of year to capture flies and so on. Therefore, one would ultimately get to the black fly PCR end-point but would not risk missing important entomological indicators such as biting rate and peak transmission season.

There was general agreement with most of the materials presented, although it was noted that there were some grey zones that needed further discussion. Whether testing adults should be removed as an option or if there should be change from PCR tests on black flies to entomological evaluation remained questions, given that positives by PCR tests were a certain indication of the need to treat, while negative tests led to uncertain outcomes.

Serological tests on adults leading to many positives results indicate that there was transmission at some point, it was stated. It was considered also that as testing adults is easier, it should remain in place. It was felt also that context must play an important part in strategy in order to fully take into account considerations about breeding sites in an area, and the presence of hyper-, meso-, or hypo-endemic areas in the same district.

On the negative blackfly PCR tests, it was noted that details would need to be added to the document in order to add specifics around negative tests; factors such as very low fly catch, coming in below transmission ATP.

It was also noted that a district approach still has some inherent pitfalls for decisions regarding stopping onchocerciasis treatment.

2.3. Recommendations

The decisions arising from the group’s discussions and endorsed by the meeting were as follows:

- In situations where black flies test negative after five rounds of MDA, it is recommended that another black fly PCR be repeated before taking a decision to stop MDA.
- There is need for operational research to establish whether five rounds of MDA are enough to eliminate transmission.
- If PCR testing is positive, continue treatment.
- Keep testing adults as an option.
- In the co-evaluation of TAS1/OEM, there is a need for OTS to make this more specific to guide programmes in proper decision-making.
• There should be more field studies in the co-evaluation strategy to generate data from a variety of geographical settings in order to support proper decision-making regarding stop-MDA.

2.4. Country feedback

Feedback on onchocerciasis elimination efforts in African countries was received from two countries: Uganda and the Democratic Republic of the Congo.

Uganda

The national onchocerciasis elimination Programme Manager raised issues related to PES. He informed the committee that most of the foci in the country have completed PTS and are to transition to PES. However, even in foci where PTS has been completed, the frequency of surveillance in these respective foci is not very clear to the programme. He cited one focus in eastern Uganda (Mt Elgon) where interruption was achieved in 2010, but to date no assessment of any kind has been conducted. This is of concern to the programme since there could be recrudescence or vector repopulation without the programme knowing. Even in those foci that are supposed to be under PES, the programme is unsure of the frequency of surveillance activities. He pointed out that without WHO guidance, partners are reluctant to support activities related to PES. In this respect, the programme might benefit if OTS could provide appropriate guidance in this area.

Democratic Republic of the Congo

The two main issues were:
• diagnostic tools used for mapping in hypo-endemic areas; and
• how to conduct assessment in onchocerciasis areas where MDA has not been implemented.

The discussion mainly focused on PES and how experiences from other countries, especially in the Americas, can be tapped and customized for programmes in Africa. OTS members observed that questions on PTS are now arising from several countries including in the Americas and in Ethiopia, Nigeria and Sudan. It is high time OTS began to think about presenting a series of recommendations to help programmes, it was stated, as there will not be much money to support PES activities. Professor Unnasch, however, observed that PES should be integrated in the individual country surveillance system for it to be sustainable; this could be reasonably inexpensive. He cited studies in the Ecuadorian focus and Mexico. In the former, Ov16 ELISA was used for surveillance while for the latter PES was integrated with the health system where the onchocerciasis brigade, under a strong national union, conducted nodule palpation, nodulectomy and follow up with Ov16 ELISA serology, checking all the children. No evidence of recrudescence was found. Similarly, in Uganda in the Victoria Nile focus, where DDT application was used in the 1970s to achieve elimination of the vector, comprehensive studies were recently conducted, as recommended by the Uganda Onchocerciasis Elimination experts’ advisory committee, to check for any possible recrudescence or vector repopulation. Entomological and serological surveillance were undertaken, but the results did not indicate any recrudescence or vector repopulation in the focus. From these examples, it was suggested that OTS should consider proper PES procedures that can be incorporated in the revised WHO guidelines for verification of onchocerciasis elimination.

2.5. Recommendations

1. There is a need for OTS to start addressing issues of PES in subsequent meetings in order to present proper procedures on PES to guide programmes and for incorporation in the revised WHO guidelines for verification of onchocerciasis elimination.

2. There is a need for countries to integrate PES into the ongoing national health surveillance system for it to be sustainable.
3. Onchocerciasis molecular black fly diagnostic initiative

In an effort to improve diagnostic tools for onchocerciasis elimination, WHO is moving forward to foster development in this area. The Diagnostic Technical Advisory Group for NTDs was established by WHO in 2019, consisting of 12 members and 20 observers. The mandate of the committee was to define TPPs based on programme needs.

The process started by reviewing programme needs, drafting TPPs and supporting stop-MDA decisions, for which WHO is making a considerable contribution. It is envisaged that a standardized nucleic acid amplification test for routine use in onchocerciasis elimination will advance the elimination process. Global Health, in collaboration with the NTD department, has launched an initiative with the goal of developing a diagnostic tool suitable for routine use, and which would be reliable and affordable. The strategy was to engage expert laboratories and, in this initiative, Professor Unnasch’s laboratory at the University of South Florida, Dr Nutman’s laboratory at the National Institutes of Health, Dr Fischer’s laboratory at Washington University in St Louis and Professor Williams’ laboratory at Smith College were all involved. The work primarily involved the comparison of sensitivities and specificities of qPCR tests, by comparing the best available assays on pools of 100 black fly heads. The three qPCR assays compared for their sensitivity and specificity were O-150 from Nutman, O-150 from Williams and ND5 from Fischer (adapted from Hendy et al.). All the laboratories tested the three assays on pools of 100 blackfly heads with a single *Onchocerca* larva added. The species of fly used was *Simulium vittatum*, a non-onchocerciasis vector reared in the laboratory.

The results of this comparative testing showed that the NIH O150 assay was the most sensitive (although all three assays were very sensitive).

In order to reliably detect one L3 larva in 100 black fly heads, it is important to have very high sensitivity because there is a lot of biological material that can interfere with assay performance.

The following characteristics of the comparison study were noted:

- In the detection of one L3 in 100 black fly heads, there was 100% agreement in all three laboratories. All three qPCR assays detected all 10 L3 containing pools.
- All three qPCR assays gave excellent sensitivity, but NIH O-150 and SC O-150 gave the best sensitivity. Overall, NIH O-150 was somewhat most sensitive.
- Specificity was assessed using *O. ochengi* DNA. There was some cross reactivity with both O150 qPCR assays. No cross reactivity was observed with the ND5 assay, which may result from its lower sensitivity.

3.1. Discussion

The subsequent discussions covered a range of issues including OTS endorsement/guidance to programmes on this new qPCR test, sensitivity and specificity of the new test and the costs involved in the diagnostic initiative. When comparing the classical Unnasch PCR-ELISA method and the new black fly/*O. volvulus* qPCR method as described by Steven Williams, it was noted that the sensitivity and specificity of the tests were similar, with a small increase in sensitivity of the new qPCR method. This new qPCR assay has a streamlined protocol and is easier to perform than the Unnasch PCR-ELISA. Additional advantages include a lower cost and decreased likelihood of amplicon contamination because the PCR plates are sealed throughout the entire PCR process. Further considerations included the fact that the new assay is targeting the same DNA sequence (O-150) and so sensitivity and specificity of the new assay are similar. Some additional validation will be needed to ensure, given the enhanced limit of detection, that only larvae are being detected and not degraded DNA from microfilariae.

The initial cost of the new qPCR approach will be higher because the qPCR instrument
costs US$ 15 000−20 000. The cost to run the new assay, however, will be lower because the necessary reagents are cheaper. Additionally, the qPCR plates can be mass-produced with the reagents pre-loaded, then freeze-dried and shipped with no cold chain. Once the plates are sealed, they are never reopened, so there is also a much lower chance of DNA contamination. This will mean retesting fewer samples and therefore reduced costs.

One important consideration in the black fly testing is the need to understand the difference between signal generated from the detection of L3 larvae and signal generated from the detection of free DNA, in order to ensure that this testing constitutes transmission monitoring rather than xenomonitoring. This issue can be addressed by limiting the number of PCR cycles and defining an assay cut-off.

Results from qPCR assay are available in 75 min, whereas the Unnasch PCR-ELISA protocol takes about 36 h.

There will also be lower labour costs because the protocol is both simpler and faster to perform than the previously used Unnasch assay. Standardization of testing will also be improved because plates will be centrally prepared with standardized, shelf-stable, freeze dried reagents and controls. Because of the stability of reagents and controls, shipping at ambient temperature will dramatically reduce programme costs. Thus, it was suggested that this would be the appropriate direction to follow in the era of onchocerciasis elimination.

The roll-out plan for validation and production was discussed; however, there was an assurance that the Williams’ laboratory at Smith College will take responsibility for producing and shipping the plates with all reagents and controls. Quality assurance and training still need to be defined, although the Williams’ laboratory has offered to provide both quality assurance and training.

Based on the work of the Williams’ laboratory for the Bill & Melinda Gates Foundation DeWorm3 soil-transmitted helminthiasis programme, the onchocerciasis qPCR assay has been designed with DNA extraction and PCR amplification controls. These controls will ensure that negatives are true negatives and positives are true positives. The controls also ensure that the qPCR is functioning within appropriate parameters. For DeWorm3, this method is being used on 250 000 stool specimens screening for four soil-transmitted helminth parasites.

Building an exogenous control into the assay system needs significant consideration. There has been much discussion about using B. malayi or B. pahangi L3s rather than O. volvulus L3s since the Brugia parasites are easy to obtain in large quantities from the FR3 at the University of Georgia, while O. volvulus L3s are extremely difficult to obtain in the numbers that would be required.

### 3.2. Recommendations

The principal recommendations were as follows:

- **NIH O150 (qPCR) should be the primary assay and OvND5 should be used as an independent confirmatory assay, in parallel, rather than in multiplex.**

  - It presents opportunities for integration with xenomonitoring work for other diseases (e.g. LF).

  - There is decreased risk of contamination from amplified PCR products because the qPCR plates are sealed before there is any amplification; plates are never opened so there is no chance of amplicon contamination of the laboratory or other plates.

  - Costs to run qPCR are lower after the initial purchase of the required qPCR instrument.

- **All reagents should be supplied to laboratories in centrally produced, pre-prepared, freeze-dried 96 well plates.**

- **The silica-based DNA extraction and purification method was efficient for isolating DNA from the black fly pools and should therefore be standardized in forthcoming standard operating procedures.**

Next steps based on these findings included the need to develop standard operating procedures and training plans (including laboratory validation and a quality assurance/quality control system) and the need to define use cases for onchocerciasis transmissions monitoring using qPCR.
4. RDT including update from Mologic

There have been four biomarker approaches for onchocerciasis and the aim was to invest in four new pathways to get better biomarkers. Collaborating institutions in this investment are the United States Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH), the Washington University in St Louis and the Expanded Special Project for Elimination of Neglected Tropical Diseases of WHO’s Regional Office for Africa (ESPEN/WHO). There are four principal biomarker approaches.

4.1. Serum immune Repertoire Approach

CDC is working on a novel antibody-based biomarker. In this a total of 22 target proteins for IgG, 18 for IgG1 and 11 for IgG4 were identified. What remains is to develop assays based on the newly discovered biomarkers to help in stop-MDA for onchocerciasis. This can be used for surveillance activities also.

- The initial phase of this project focused on work with Serum immune to identify candidate peptides and peptide for onchocerciasis, LF and schistosomiasis. The project is now moving to phase 2 where the candidates will be put into peptide arrays before developing assays. The hope is that there will be more to report in the next 6 months.

4.2. Stage-specific Protein Microarray Approach

NIH is pursuing a novel antigen and nucleic acid-based approach for discovering new biomarkers. This antigen marker qualification, where multiple independent laboratories are involved in validation of ELISA for detection of microfilaricidal markers, tests for presence in patent infections and their disappearance after microfilaricidal drug administration. Also prototype lateral flow RDTs based on marker and antibody optimization, and community input and alignment around marker validation planning and execution.

4.3. Immuno-precipitative “Multi-omics” Approach

Washington University is looking at a novel antigen-based biomarker for active infection. This will involve identification and confirmation of panels of proteins as targets for antigen detection of viable adult *O. volvulus* worms. This will target development of a sensitive and specific antigen detection assay (or assays) that meet TPPs and can be used to assess worm viability in the laboratory or in the field, in addition to evaluating antigen detection assays for viable adult *O. volvulus* using pre-and post-treatment samples (ivermectin, doxycycline).

4.4. CFDNA/CFRNA sequencing (onchocerciasis and LF)

This will involve independent verification of candidate cfDNA and cfRNA using archived plasma samples; and testing in both plasma and urine samples before and after microfilaricidal therapy. In LF this will be done pre-and post-MDA and pre/post doxycycline administration, while for onchocerciasis, it will be during doxycycline or other microfilaricidal therapy.

Christy Hanson then discussed the optimization of the Ov16 RDT (Mologic and Bioaster), stating that the grant from the Bill & Melinda Gates Foundation would be extended to cover the development of an improved ELISA kit. She also noted that tests would need to be conducted based on the performance characteristics and costs defined in the new onchocerciasis TPPs, the hope being that there would be a prototype for laboratory validation at the CDC by June 2021. It would then be sent out for field validation.

4.5. Discussion

The discussion centred on plans to develop a replacement diagnostic kit now that the Ov16 ELISA produced by a Korean company is no longer available.

It was confirmed that Abbott had ceased production and sale of ELISA kits, although it had been possible to source a few for comparison purposes. Development of an ELISA kit had been added to the scope of the Mologic grant, but until then countries would need to rely on the OEPA ELISA.
5. DBS on RDT protocol update

Work on this topic has been ongoing since the last OTS meeting.

Since the last meeting, three laboratories (Cameroon, Kenya, CDC) have worked to generate data for DBS on RDT performance. However, data presented from various field studies in Malawi, Togo and the United Republic of Tanzania revealed that, while there is variable good performance of DBS RDT in different settings, in all the settings DBS RDT performed better than RDT. There were also issues with the specificities of the DBS and three laboratories were engaged to conduct additional studies. These studies had positive and negative DBS panels.

In the positive DBS panel, 80 samples were used from the Democratic Republic of the Congo (12), Ethiopia (26), Uganda (28) and the United Republic of Tanzania (14). This positive panel was constructed using parasite-confirmed sera and negative red blood cells from uninfected donors. The negative panel was assembled using field-collected DBS from non-onchocerciasis endemic countries. A total of 99 samples were used from the Philippines (50) and Saint Lucia (49). Both the DBS panels were shipped to participating laboratories and tested by SD ELISA and DBS on RDT. However, SD ELISA could not be performed in Cameroon due to limited negative DBS.

5.1. Results of positive panel performance

The results of the positive panel performance were as follows:

- 71/80 samples positive, both tests in all the laboratories. However, two samples were negative in all the laboratories.
- All laboratories had good concordance; ELISA detected most positives (95–98% sensitivity).
- RDT identified similar proportion of positives, but lower sensitivity in Cameroon (91%).

In the negative panel the results were:

- 84/99 samples were negative by all tests in all the laboratories.
- SD ELISA was specifically lower at CDC (85%) than in Kenya (97%); however, the CDC ELISA was consistent with what was presented at the third OTS meeting.
- RDT specificity ranged from 96% to 100%.

The meeting was assured that there had been good concordance overall between the laboratories. The ELISA had detected most of the positives, but the RDT had picked up a similar proportion of positives, even though the sensitivity was slightly lower in the Cameroon laboratory.

Key messages

- All three laboratories showed comparable results on both panels.
- The performance of DBS on RDT appears to have comparable sensitivity to SD ELISA, but is slightly lower in Cameroon.
- RDT run from DBS appears to perform better than SD ELISA on specificity; RDT from DBS may alleviate concern regarding false-positive tests.
- Imperfect specificity of DBS on RDT method should be taken into consideration when used in programme settings.

5.2. Discussion

In the discussion following the presentation, the first question was about specificity with false positives: would one simply run the test multiple times if one were to get an inconclusive result? There was confirmation of the need to run the test multiple times; however, in this case you would take a best of three score. This also assumes that the tests are independent. If there is a problem with cross reactivity with another worm, it is likely that positives will show in all three tests.

On the difference between CDC and Cameroon tests, Kim Won said the reason for this was not clear. The study had tried to keep everything as similar as possible: negative blood spots were field collected; they were from the same disk but were different spots. The kits were also from the same lot.
The response was similarly inconclusive to a question about the variation in SD Bioline kits between different laboratories. Here again, everything was kept as consistent as possible but only so much can actually be controlled. It would be helpful to know why the differences exist, but that, given the uncertainty about the availability of kits, the focus should shift to making better kits. Where there are new kits to be used, it was noted, there would be things to learn from the old kits to ensure that the next generation of kits does not have the same problem.

A concern was raised about degrees of confidence in the 98% specificity value. Given a requirement to measure 2%, the usefulness of the RDT would vary significantly between 96% and 98% specificity. The differences in the SD Bioline from the three laboratories has no clear explanation as it stands, but CDC had a focus to identify a much better diagnostic kit, learning from this experience. On the 98% specificity, CDC reiterated that this is better than ELISA based on the results and bearing these points in mind. Despite this, there is still some need to evaluate the specificity. It was recognized that during the study, the positive and negative panels were quite limited, and some would also be preserved for future use.

Given the data generated, this should constitute a starting point and be used to move forward. There is a need to evaluate in real time as information comes in and even where the lower end of the specificity to be chosen, it would be possible to adjust the decision algorithm to account for it. Instead of < 4, it would be 6. There would not in fact be a huge increase in numbers if specificity were 96%.

In response to a question about practical recommendations by the group, the answer came that DBS be taken to the field only for mapping. There was a recommendation also that ELISA is not necessary: simply elute the DBS with the buffer to run the RDT in the laboratory.

5.3. Conclusions

Performance of DBS on RDT had comparable sensitivity to the SD ELISA, while specificity was higher than the SD ELISA.

Available resources

The following resources are available for programmes:

1. A recommendation was made for buffer preparation, use and storage, namely that an elution buffer is required for DBS on the RDT method. The buffer was originally available from PATH, but a comparison study revealed that it can in fact be produced locally in the labs.
   - Buffer recipe and bench aid for DBS on RDT method RDT buffer preparation, use and storage. This information can help laboratories performing DBS on RDT to prepare buffer.

2. RDT Buffer preparations, use and storage procedures. This provides information on making buffer and all the necessary steps. Also provided are standard operating procedures for DBS on RDT.

3. Training video [in progress]. Virtual training was pilot tested in the United Republic of Tanzania in October 2020. All the training materials will be updated based on the Tanzanian experience.

A reflection on the third OTS meeting regarding Stage 1 OEM was considered. The focus was on tests using eluted DBS on RDTs, if programmes have insufficient experience with ELISA. Ways of eliminating false-positives were then proposed in algorithms to help laboratories work through. It was felt that some kind of decision can be made with the use of this tool. The proposed decision algorithms could help, whether the results were positive or inconclusive. In this case, if the result is yes, the area is classified as endemic and MDA should be initiated. However, if the final result is No, or the results are inconclusive, one might then proceed to Stage 2 OEM. This takes into consideration imperfect specificity.

DBS on RDT for stage 1 mapping, given imperfect specificity, was proposed as follows, with a proposed testing algorithm to help repeat testing, if possible, in order to arrive at a conclusion/decision:

- green boxes = interpretation can be made
- red boxes = interpretation needs further action
- yellow boxes = inclusive

This system is intended to help laboratories decide if a DBS result is positive, negative or inconclusive.

A decision-making algorithm was also proposed when results are available, for the first stage of mapping, assuming 98% specificity:
If there are fewer than four positive or inclusive results, make a decision.

- If the answer is Yes, first-line villages pass stage 1 and stage 2 OEM is recommended.
- If the answer is No, look at village level results. If any village has nine or more positives, consider the area endemic and proceed to MDA. If not, proceed to stage 2 mapping.

5.4. Recommendations

1. To take DBS into the field, do not use ELISA because the results are inconsistent.
2. Programmes should use RDT run from DBS because it is easy to perform.
3. There should be independent confirmatory tests to validate ELISA.
6. DTAG onchocerciasis subgroup update

The DTAG was assembled by WHO in October 2019 and consists of 12 members and around 20 observers. The subcommittee with expertise in onchocerciasis was tasked with defining the ideal profile of new diagnostics and to contribute to the definition of TPPs based on programme needs.

It was confirmed to OTS that TPPs were currently being developed in two specific fields relevant to the OTS’s scope:

- **Mapping in hypo-endemic areas**
  - > 2% prevalence threshold
  - 60% sensitivity, 99.8% specificity
    - Based on acceptance of undertreating 5% of the time
  - RDT preferred, but will accept a laboratory-based test

- **Stopping MDA**
  - < 1% prevalence threshold
  - 89% sensitivity, 99.8% specificity
    - Based on over treating 10% of the time
  - RDT preferred, but will accept a laboratory-based test

It should be noted that the 2% and 1% thresholds are based on 2019 OTS recommendations, which were made in the context of serological (antibody) assays. The prevalence threshold was used to calculate the required sensitivity and specificity, and specificity based on work by Dr Katherine Gass. This therefore implies that for individuals:

- if the person is truly infected, the test results should be positive, a% of time (sensitivity); and
- if the population is uninfected, (< 2% threshold), the cluster survey must show negative y% of the time

The x and y values were calculated by Dr Gass.

For mapping (2% threshold): 60% sensitivity and 99% specificity, while for stopping (1% threshold), 89% sensitivity and 99.8% specificity. The key features of the two TPPs are: for mapping, it must detect 2% of the analyte prevalence, 60% sensitivity and 99.8% specificity; and for stopping, it must detect 1% of the analyte prevalence with 88% sensitivity and 99.8% specificity.

6.1. Main requirements for an ideal RDT

The ideal RDT would need to have the following characteristics:

- targets all ages of individuals resident in a defined geographical area;
- test can be performed under zero infrastructure conditions including but not limited to health centres, households and outdoors;
- test can be performed by health workers and community health workers;
- training requirements: one day for community volunteers and lay persons;
- no maintenance or calibration required;
- the sample volume of whole blood 5–15 µL;
- stable for 36 months at 4–40 °C and can tolerate excursion to 50 °C for 2 weeks; and
- calometric or other indicators to identifying excessive heat/humidity exposure.

6.2. Discussion

In the ensuing discussion, it was suggested that consideration be given to the confirmatory test approach. Current WHO guidelines allow use of skin-snip PCR as a confirmatory test for Ov16 serological tests, but this can be problematic because such a
test is less sensitive than serology. What would a confirmatory test look like, therefore?

In response, it was stated that sensitivity is indeed lower, but skin snip PCR should be extremely specific. If the sensitivity standard is set at 60%, up to 90% sensitivity can be achieved, which provides a buffer that allows one to conduct skin snip PCR. Beyond that, there had been no in-depth consideration of the nature of a confirmatory test.

It was confirmed also that TPPs would be shared with group members via email and posted online. It was stated that they ought to be available within the next 2 weeks (from the date of this meeting) for a consultation period of 28 days. It was recommended that TPPs be shared as widely as possible so that all concerned parties have a chance to provide input.

6.3. Conclusion and next steps

TPPs for mapping onchocerciasis in hypo-endemic areas and stopping MDA were drafted. One key feature is that regardless of the application, the tests must be > 99.8% specific. The sensitivity needs to be > 60% for mapping and > 89% for stopping MDA.

A series of topics to consider next was then laid out:

- How to demonstrate such high specificity? How do you assemble a sufficiently large panel with enough representative geographies/coinfections so that developers can demonstrate such high specificity?
- The monitoring and evaluation use case.
- Historically there have been different tests, which complicates the analysis of results. Using the same tool for each use case would be ideal, but, if this is not feasible, we need a strategy to compare results over time across different tools.
- The thresholds used were based on the assumption of using antibody-based tests. Thresholds would be different for antigen-based tests. What impact would a new threshold have on the sensitivity and specificity requirements?
- If Ov16 meets the performance requirements, is skin-snip PCR acceptable for a confirmatory test?
- How to proceed in *Loa loa* co-endemic areas?
7. Update on the entomological manual for programme managers

The committee then received an update from Professor Bertram Nwoke, who is responsible for drafting the *Entomological manual for onchocerciasis elimination programmes*. Professor Nwoke was assisted in this work by three colleagues with expertise in medical entomology and parasitology: Dr M.A. Adeleke, Professor H. Mafuyai and Professor K.N. Opara.

The manual consists of 12 chapters in all entomological areas that are relevant to onchocerciasis elimination, as per the Contents list:

- Chapter 1: Introduction
- Chapter 2: General characteristics and life cycle of black flies
- Chapter 3: Breeding sites of black fly vectors
- Chapter 4: Identification of black fly vectors in Africa
- Chapter 5: Selection of study sites and community mobilization for entomological evaluation
- Chapter 6: Prospection of breeding sites of black flies
- Chapter 7: Collection and preservation of black fly larvae for cytotaxonomical identification
- Chapter 8: Human landing collection and trapping of female black flies for entomological evaluation
- Chapter 9: Dissection of female black flies to determine parity and infectivity
- Chapter 10: Preparation of female black flies for polymerase chain reaction pool screening assay and interpretation of results for onchocerciasis elimination.
- Chapter 11: Determination and interpretation of transmission indices in onchocerciasis elimination
- Chapter 12: Black fly control in onchocerciasis elimination programmes

Professor Nwoke informed the meeting that the first draft of the manual has been completed and was subjected to editorial review. Some corrections have been made, leading to the second draft. The second draft was completed and forwarded to Dr Paul Cantey. WHO Geneva constituted a committee of experts that is currently reviewing the draft manual; 8 of the 12 chapters were reviewed during a zoom meeting held on 12–15 October 2020. The remaining chapters will be reviewed during a two-day meeting to be held on 2–3 November 2020.

7.1. Discussion

The subsequent discussion focused on the mechanisms for updating some chapters in the manual to include some innovative vector control strategies such as “slash and clear”. Professor Nwoke confirmed that the manual could be updated as new evidence became available, to include, for example, details of the qPCR methods discussed in the ongoing OTS meeting.

Regarding the intended use of the publication, it was stated that while it was a document which would, of course, be of interest to scholars and specialists, the current draft was oriented for use by people in the field. In the review process, it was noted, it would help to ensure that the manual becomes a tool for use in the field. Discussion of the manual focused on what was needed by programmes to do what they need to do, because it was observed that the document was devoid of practical considerations of the reality on the ground. It was reiterated, however, that during the final day of the review, there would be a discussion about ensuring that training materials and bench aids are ready to take to the field.

There was confirmation also that the manual will be posted as a PDF online, but that WHO would need to think about ensuring that some of these manuals are more App friendly. It was noted that the skin NTDs group has experience in such work.
It was observed that training in the use of this manual would be crucial in its roll out, and this remains to be worked on. There was an observation on the need to speed up and get the manual out, as this is critical for programmes. In the era of COVID-19, some countries (for example, Uganda) have pilot tested virtual training, which has been successful and could be adopted for rolling out this manual.

It was also confirmed that plans are already afoot to translate the document into French. Possible translation into Portuguese would require verification.

7.2. Recommendations

1. The current review of the entomological manual, involving specialists in the various fields, should take into consideration the application of the document in the field, and include areas such as handling of PTS in appropriate sections.

2. WHO should endeavour to make the manual available for field use as soon as possible in order to support country programmes in their elimination efforts.

3. WHO should plan to translate the manual into French and Portuguese as soon as it becomes available to prevent Francophone and Lusophone countries from lagging behind.
The key recommendations on qPCR are as follows:

- Continue operational research on qPCR.
- OTS should support eventual adoption of real time platform for O-150 with ND5 as the confirmatory assay.
- Operational research is needed: compare new methods with old methods to ensure that the lower limit of detection in this assay will not pick up on old microfilariae in the thorax rather than L3s, and adjust thresholds if necessary.

- WHO guidelines as written only specify O-150 PCR, so if the main recommendation here is to use O-150 with ND5 as the confirmatory assay, it should be easy to add with endorsement from OTS.
- The requirement for a confirmatory test might require a new review process. Changing from PCR ELISA to qPCR should be relatively easy.
- There is a need to get interim qPCR guidance out to countries.
- OTS report to mention strategies for interim guidance as new technologies become available.
## Annex 1. Agenda

### Day 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Item</th>
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<tbody>
<tr>
<td>I. Opening session</td>
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<tr>
<td>15:00–15:05</td>
<td>Welcome and introductions</td>
<td>WHO</td>
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<tr>
<td>15:05–15:10</td>
<td>In memoriam – Ricardo Thompson</td>
<td>WHO</td>
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<tr>
<td>15:10–15:15</td>
<td>Review of meeting objectives</td>
<td>Chair</td>
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<tr>
<td>15:15–15:30</td>
<td>Highlights of the third meeting</td>
<td>Chair</td>
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<tr>
<td>II. Evaluation</td>
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<tr>
<td>15:30–15:45</td>
<td>Co-evaluation of onchocerciasis and LF</td>
<td>Paul Cantey</td>
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<tr>
<td>15:45–15:55</td>
<td>United Republic of Tanzania: experience in the field</td>
<td>Upendo Mwingira</td>
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<tr>
<td>15:55–15:25</td>
<td>Discussion</td>
<td>ALL</td>
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<tr>
<td>III. PCR comparisons</td>
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<tr>
<td>15:25 – 15:45</td>
<td>PCR comparison work</td>
<td>Steve Williams</td>
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<tr>
<td>15:45–16:00</td>
<td>Discussion</td>
<td>ALL</td>
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### Day 2

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<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>IV. Other updates</td>
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<tr>
<td>15.00–15.30</td>
<td>Country Feedback &amp; Concerns</td>
<td>Countries – Closed</td>
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<tr>
<td>15:30–15:45</td>
<td>Rapid diagnostic tests including update from Mologic</td>
<td>Christy Hanson</td>
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<tr>
<td>15:45–16:00</td>
<td>Dried blood spot elution protocol</td>
<td>Kim Won</td>
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<tr>
<td>16:00–16:15</td>
<td>Target product profile updates</td>
<td>Marco Biamonte</td>
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<tr>
<td>16:15–16:30</td>
<td>Draft entomological manual for onchocerciasis elimination programmes</td>
<td>Bertram Nwoke</td>
</tr>
<tr>
<td>16:30–17:00</td>
<td>Discussion and wrap up</td>
<td>ALL</td>
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</tbody>
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Annex 2. List of participants

Members
Dr Paul Cantey (chair), United States Centers for Disease Control and Prevention, Atlanta, USA
Professor Thomas Unnasch, University of South Florida, Tampa, USA
Dr Katherine Gass, The Task Force for Global Health/NTD Support Center, Decatur, USA
Dr Robert Klein, Universidad del Valle de Guatemala, Guatemala City, Guatemala
Dr Joseph Kamgno, University of Yaoundé, Cameroon
Mr Thomson Lakwo (rapporteur), Onchocerciasis Control Programme (retired), Entebbe, Uganda
Dr Upendo Mwingira, National Institute for Medical Research, Dar-Es-Salaam, United Republic of Tanzania
Professor Asam M.A. Zarroug, Federal Ministry of Health, Khartoum, Sudan

Invited experts
Dr Marco Biamonte, Drugs & Diagnostics for Tropical Diseases, San Diego, USA
Dr Christy Hanson, Bill & Melinda Gates Foundation, Seattle, USA
Dr Thomas Nutman, National Institutes of Health, Bethesda, USA
Prof Bertram Nwoke, Imo State University, Owerri, Nigeria
Dr Sharon Roy, United States Centers for Disease Control and Prevention, Atlanta, USA
Dr Kim Won, United States Centers for Disease Control and Prevention, Atlanta, USA
Dr Martin Walker, Royal Veterinary College, Hatfield, United Kingdom
Dr Louise Hamill, Sightsavers, Haywards Heath, United Kingdom
Professor Daniel Adjei Boakye, University of Ghana, Accra, Ghana
Dr Charles Mackenzie, Task Force for Global Health, Atlanta, USA

Secretariat
Ms Junerlyn Farah Agua, Strategic Information and Analytics, Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
Ms Camilla Ducker, Consultant
Mrs Lakshmi Jonnalagedda, Prevention, Treatment and Care, Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
Dr Jonathan King, Prevention, Treatment and Care, Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
Mr Ashok Moloo, Strategic Information and Analytics, Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland
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