Federal Democratic Republic of Ethiopia
Ministry of Health

GUIDELINES FOR ONCHOCERCIASISELIMINATION IN ETHIOPIA

July 9, 2015

Addis Ababa, Ethiopia

PREFACE

Human onchocerciasis (river blindness) is one of the debilitating neglected tropical diseases (NTDs) that have drawn the attention of national governments, non-governmental organization, pharmaceutical companies, philanthropists and health development practitioners worldwide. Human onchocerciasis is an eye and skin disease caused by a filarial worm called *Onchocerca volvulus*, which is transmitted by the bite of an infected black fly, of the genus *Simulium*. These flies breed in fast-flowing streams and rivers, hence the name "river blindness." People with infections usually have severe itching, dermatitis, eye lesions, and/or subcutaneous nodules. In most of the endemic countries, blindness is inevitable for those with severe and chronic infections, though this is not the case in Ethiopia. The disease is one of the underlying causes of poverty amongst the communities where it is prevalent.

The World Health Organization (WHO) estimates that approximately 37.2 million people are infected and 1 million blinded or are visually impaired in 38 endemic countries around the world. Nigeria is known for its highest endemicity in the world followed by Ethiopia and the Democratic Republic of Congo. More than 20 million people live in the surveyed endemic areas of Ethiopia and are affected by the disease or are at risk of infection. Studies conducted so far indicate that the disease is mostly found in southwestern, western and northwestern parts of the country..

The development of the Ethiopian National Guidelines for Elimination of Onchocerciasis arose out of a felt need to implement programs geared towards transmission interruption using standard criteria for verifying claims of local interruption of onchocerciasis transmission which would form the basis of cessation of intervention and pave way for verification of elimination. Its development was based on WHO guidelines on verification of elimination of transmission of *O. volvulus* onchocerciasis and experiences acquired from the elimination of diseases like smallpox, poliomyelitis, and elimination of onchocerciasis programs in the Americas, Sudan and Uganda.

This document sets core activities to be accomplished in order to document transmission elimination which include a period of post-treatment surveillance followed by a process for verification of the country and immediate post verification interventions to check disease recrudescence or a reinvasion of the disease transmitting vectors. Despite the challenges facing the FMoH, it is envisaged that the goal of onchocerciasis elimination in Ethiopia will be achieved through concerted action by all partners under the leadership of the FMoH.

This guideline is developed by FMoH in partnership with all organizations that have been involved in the control of onchocerciasis in Ethiopia. The FMoH remains committed to the strategy of onchocerciasis elimination in Ethiopia and will put in place an effective surveillance system to monitor the disease situation during the post-treatment surveillance period in all formerly endemic areas when and where interventions have been halted.

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ACKNOWLEDGEMENTS

This guideline for the of onchocerciasis elimination in Ethiopia was developed by the Disease Prevention and Control Directorate, Neglected Tropical Disease Case Team of the Federal Ministry of Health in collaboration with World Health Organization (WHO), The Carter Center (TCC), Light For The World (LftW), USAID RTI International/Envision project, Ethiopian Public Health Institute (EPHI), Lions Clubs, Addis Ababa University College of Health Sciences School of Medicine (AAU-CHS-SoM), and Armauer Hansen Research Institute (AHRI). The Federal Ministry of Health thanks and appreciates all of these institutes for their very useful contributions during the development and finalization of this guideline.

The Federal Ministry of Health extends special thanks to all members of onchocerciasis technical working group: Mr. Oumer Shafi (FMoH), Mr. Kadu Meribo (FMoH), Dr. Abate Mulugeta (WHO), Dr. Zerihun Tadesse(TCC), Mr. Aseged Taye(TCC), Mr. BirhanuDawa (TCC), Mr. SindewMekasha (EPHI), Dr. Tamrat Abebe (AAU-CHS-SoM), Mr. Dubewak Gemeda (LFTW),and Dr. Endalemaw Gadissa (AHRI).The technical assistance of Drs. Moses Katabarwa and Frank Richards (TCC Atlanta),Dr. Rory Post (Liverpool John Moores University), and Dr. Mark Eberhard is also gratefully acknowledged.

ACRONYMS

APOC	African Program for Onchocerciasis Control
CDC	Communicable Disease Control
CDTI	Community Directed Treatment with Ivermectin
CMFL	Community Microfilaria Load
DNA	Deoxyribonucleic Acid
DTT	Dithiorythritol
ELISA	Enzyme Linked Immuno Sorbent Assay
EOEEAC	Ethiopia Onchocerciasis Elimination Expert Advisory Committee
EPHI	Ethiopian Public Health Institute
FMoH	Federal Ministry of Health
HCL	Hydrochloric acid
HDA	Health Development Army
HEWs	Health Extension workers
HSDP	Health Sector Development Program
IACO	International American Conference on Onchocerciasis
IVT	International Verification Team
MDA	Mass Drug Administration
MDP	Mectizan Donation Program
NVC	National Verification Committee
NOCP	National Onchocerciasis Control Program
OD	Optical Density
OEPA	Onchocerciasis Elimination Program of the Americas
PCR	Polymerase Chain Reaction
PES	Post Endemic Surveillance
PTS	Post Treatment Surveillance
SDS	Sodium Dodecyl Sulphate
SSPE	Saline Sodium Phosphate
STP	Seasonal Transmission Potential
WHO	World Health Organization

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1. INTRODUCTION

Human onchocerciasis, or river blindness, is a vector borne disease resulting from infection with the parasitic filarial worm, *Onchocerca volvulus*, transmitted from person to person by the bites of black flies that breed in fast-flowing rivers and streams. In the human host, adult male and female *O. volvulus* worms become encapsulated in fibrous tissue (nodules) and fertilized females produce embryonic microfilariae that migrate to the skin, where they are ingested by the black fly vectors during a blood-meal. In the vector, the microfilariae develop into the infectious L3 stage, at which time they can be transmitted to the next human host via subsequent bites. The parasite has no known environmental reservoir or important nonhuman hosts.

Over 99% of the disease occurs in Africa. Onchocerciasis is also found in six countries of Latin America and Yemen in the Arabian Peninsula. Current estimates suggest that over 123 million people are at risk of infection in 38 endemic countries; with at least 37.2 million of those infected and 1 million blinded or have severe visual impairment (Crump *et. al*. 2012). Recent reports by the African Program for Onchocerciasis Control (APOC) estimated that more than 102 million individuals are at high risk of the disease in 19 African countries in the Sub-Saharan belt extending from Senegal to Ethiopia (WHO, 2011). Infection with *Onchocerca volvulus*, produces eye lesions in some individuals, which can lead to blindness (Abiose, 1998), and also severe itching and disfiguring lesions of the skin (Kipp, *et. al,* 2002). The disease has severe socioeconomic and psychological consequences. The stigma associated with the disease may reduce marital prospects among affected individuals, disrupt social relationships, and cause loss of self-confidence. Among agricultural workers, onchocerciasis has been associated with increased time away from work and reduced productivity, leading to lower income (Alonso, *et, al*, 2009).

The main strategy for the control of onchocerciasis in endemic countries is by means of ivermectin (Mectizan®) distribution donated by Merck & Co. since 1987. When used on an individual basis and annually, ivermectin rapidly kills the microfilariae and reduces the fecundity of adult female worms, but does not kill them (Taylor *et al*., 1989; Chippaux *et al*., 1995). Repeated rounds of treatment with good coverage, can stop transmission and increase mortality in adult worms.Ivermectin mass drug administration (MDA) at the community level is used to ***control*** morbidity, and most recently has been used to ***interrupt and ultimately eliminate***

transmission. However, the frequency and duration of ivermectin MDA to accomplish elimination is influenced by precontrol endemicity (force of infection) and treatment coverage (Borsboom *et al*, 2003).

In 2013, Ethiopia declared a goal of attaining the interruption of onchocerciasis transmission nationwide by 2020. The objective of this National Onchocerciasis Elimination Guideline is to assist in this national paradigm shift from ***control*** to ***elimination*** by setting broad core activities of the Ethiopian Onchocerciasis Elimination Program, defining criteria for the four phases of the road to elimination, and establishing the process for having the national elimination formally verified by the World Health Organization.

2. ONCHOCERCIASIS CONTROL IN ETHIOPIA

2.1 Onchocerciasis in Ethiopia

In Ethiopia, onchocerciasis is a disease of poor rural populations, and it is in fact one of the underlying causes of poverty amongst the communities where it is prevalent. Italian investigators first reported the existence of onchocerciasis in southwest Ethiopia in 1939. Since then, extensive epidemiological surveys conducted by several workers have confirmed the presence of the disease, with varying degrees of endemicity, in the southwestern, western and northwestern parts of the country (Oomen, 1969; Zein, 1986, 1993; Taticheff *et al*., 1987; Gunderson *et al*., 1988). Later on Rapid Epidemiological Mapping of Onchocerciasis (REMO) conducted between 1997 and 2001, 2011 and most recently in 2012 confirmed the western zones of Oromia Region and Southern Nations Nationalities and Peoples' Region (SNNPR), the northwestern areas of Amhara Region and bigger part of Gambella and Benishangul-Gumuz regions to be hyper- and meso endemic for onchocerciasis. The infection is particularly associated with coffee growing areas in the southwest and with cotton and oil seed farming areas in the north-west. The total at risk population is currently estimated to be more than 20 million with about 5.8 million people living in hyper and meso-endemic areas, but this is certainly an underestimate. Large parts in the east still remain unmapped and comprehensive surveys and mapping of onchocerciasis transmission is might be needed to ascertain the full distribution of the disease in order to determine the actual numbers of persons at risk in Ethiopia. The current available information on the distribution of onchocerciasis is shown below in Figure 1.

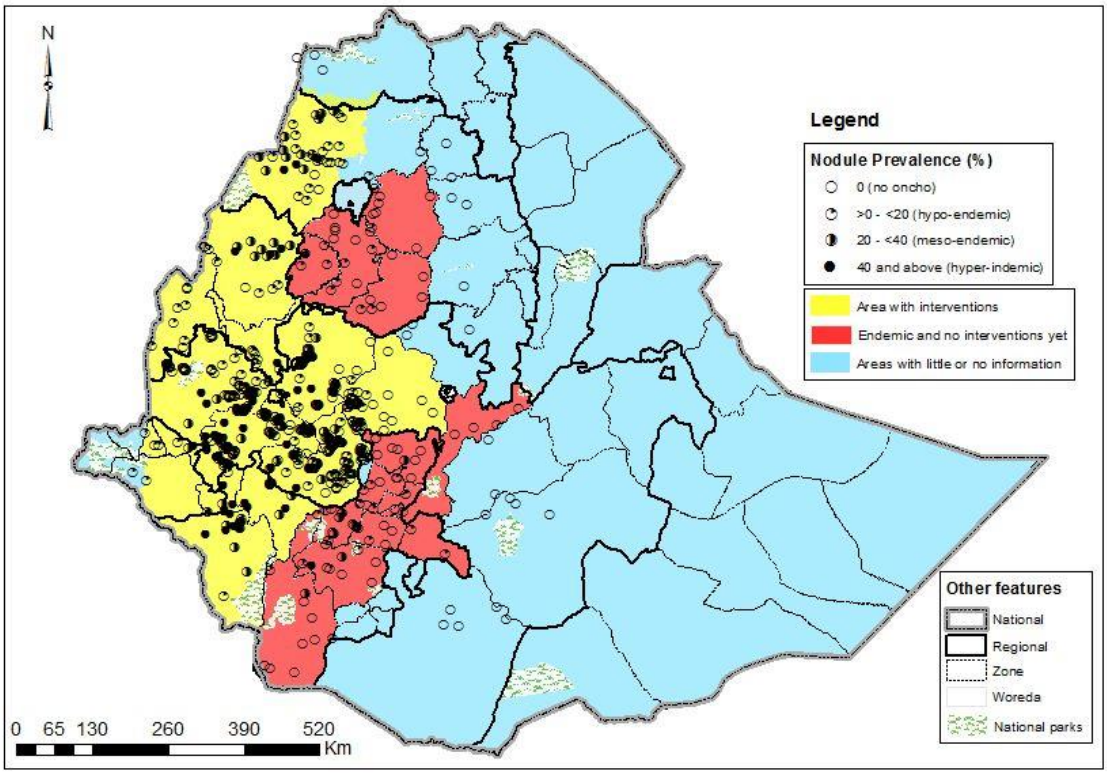


Figure 1: Status of Onchocerciasis in Ethiopia

Two black fly species groups have been identified to be vectors of onchocerciasis in Ethiopia. Flies of the *Simulium damnosum* complex, which are widely distributed throughout the endemic and non-endemic areas of the country, have long been suspected to be the major vectors of onchocerciasis in the country (Oomen, 1969; Raybould and White, 1979; Mebrahtu *et. al*., 1980).This has been confirmed by more detailed studies in some selected foci that reported natural infection with third-stage larvae of *O. volvulus* (Gebre-Michael and Gemetchu, 1996; Taye *et. al*., 2000). On the other hand *S. ethiopiense* (a member of the *S. neavei* group), which has limited distribution in the smaller rivers of southwestern midlands and the highlands and often sympatric with *S. damnosum* s.l. is suspected to play a secondary role. *Simulium ethiopiense* shows strong anthropophilic behavior but its density often remains quite low at all seasons (Raybould and White, 1979; Gebre-Michael and Gemetchu, 1996; Taye *et al*., 2000).Generally the *S. neavei* group is known to thrive best in heavily forested riverine environments and thus this decline in *S. ethiopiense* have been attributed to degradation of vegetation cover.The few attempts to isolate infective larvae resembling *O. volvulus* from *S. ethiopiense* in Ethiopia have so far been unsuccessful (White, 1977; Gebre-Michael and Gemetchu, 1996; Taye *et al*., 2000).

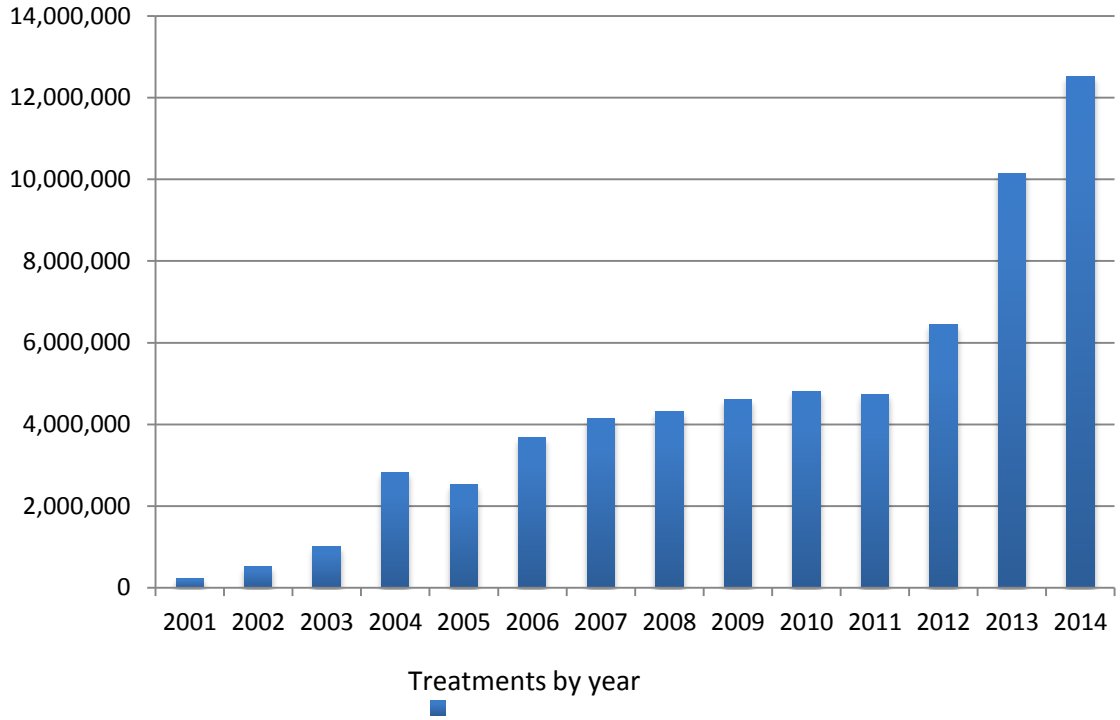
Lymphatic filariasis is another parasite that is transmitted by mosquitoes and causes elephantiasis. Onchocerciasis and lymphatic filariasis (LF) have overlapping endemicity in many parts of Africa, and Ethiopia is no exception. There is also an LF elimination effort in Ethiopia (with a similar national goal of transmission interruption by 2020) and both elimination programs use ivermectin MDA (in LF ivermectin MDA is combined with albendazole, donated by GSK). The 'stop MDA' decision for one is influenced by the transmission status for the other. Although the details of these challenges are beyond the scope of this onchocerciasis guideline, the two programs will need to work together closely if either or both are to achieve their 2020 goals. Coordination of onchocerciasis and LF elimination efforts is essential in foci where co-endemicity exists so that elimination of both diseases can be achieved in an integrated fashion, especially transmission assessments. As with onchocerciasis, mapping of LF in Ethiopia has not yet been completed.

2.2. The history of the onchocerciasis control program in Ethiopia

In Ethiopia, the control of onchocerciasis by mass treatment with ivermectin (Mectizan®) in the hyper- and meso-endemic woredas began in 2001 in the then Kaffa-Sheka Zone of SNNPR and scaled up to other parts of the country after 2004.The African Programme for Onchocerciasis Control (APOC) and other partners have focused MDA only on areas where nodule rates exceeded 20% (meaning an estimated 40% microfilaria prevalence) as these areas (called meso- and hyper-endemic areas) are those where morbidity from onchocerciasis is greatest. Areas below those MDA control threshold indices (the so called hypo-endemic areas) were not covered under the control strategy (Katabarwa et al, 2008).

The onchocerciasis control program in Ethiopia has been running for the last 12 years with technical, financial and logistic support from The Carter Center/Lions Clubs, APOC and Light for the World (LftW). Currently there are 18 community-directed treatment with ivermectin (CDTI) project zones in the country. Millions of treatments of ivermectin have been provided in Ethiopia between 2001-2014 (Figure 2).

Figure 2: Number of Ivermectin Treatments in Ethiopia, 2001– 2014



Despite success of the control program, there are major challenges: 1) the possible requirement of indefinite annual distribution of Mectizan® (Richards et al., 2000; Winnen et al., 2002); 2) uncertainty regarding possible future drug resistance (Osei-Atweneboana et al, 2007); 3) donor fatigue for a program that has no end in sight; 4) assumption that government policy can be implemented indefinitely in the face of limited resource and ever present danger of loss of knowledgeable and experienced program personnel resulting from transfer from the program or retirement.

Recent data in southwest Ethiopia (Sheka and Jimma) have demonstrated that despite8-10 years of annual Mectizan distribution, microfilarial rates average an unacceptable 6.9% (range 0%-15.2%). Importantly, children under ten years of age were found harboring mf in skin biopsies that show the existence of new infections and therefore conclusive evidence of recent ongoing transmission. However, the epidemiological picture in the country is mixed, since an evaluation conducted in North Gondar (from Metema and Quarra woredas) revealed absence of mf but presence of seropositive children. Thus, transmission could potentially be interrupted in that area.

3. ELIMINATION OF ONCHOCERCIASIS IN ETHIOPIA

In 2013 the Federal Ministry of Health (FMoH) of Ethiopia released a new master plan for the Neglected Tropical Diseases (NTDs) that included a change in policy from onchocerciasis control to onchocerciasis elimination by 2020.As part of this policy change, a guideline for criteria of elimination in the country was developed, and a new committee, the Ethiopia Onchocerciasis Elimination Expert Advisory Committee (EOEEAC) was proposed to guide the FMoH elimination program**.**

The rationale for the national onchocerciasis elimination guidelines is to assist the program and the EOEEAC steer the paradigm shift in the country from control to elimination, and establish a WHO acceptable standard for verifying national elimination. The development of the guidelines herein are based on the WHO Geneva 2001 approved guidelines, and/or the 2013 WHO/NTD Strategic and Technical Advisory Group (STAG) approved (but still draft guidelines), with consideration of the experiences of the Americas (OEPA), APOC, Sudan, and Uganda. These guidelines are therefore meant to assist the government and the committee to monitor, evaluate and document the impact of interventions on the process of elimination of onchocerciasis in Ethiopia.

3.1 Transmission interruptionby 2020 is the goal

The goal is to interrupt transmission of *O. volvulus* from the entire country of Ethiopia by 2020, and stop interventions. By 2020 the country will be in various stages of post treatment surveillance (PTS) and will have constituted a National Verification Committee active in developing a detailed country dossier to be submitted to WHO to initiate a process of formal verification of elimination.

3.2 Annual and semi-annual treatments, as well as other tools

Annual and semi-annual (every six months) MDA with ivermectin will be the main strategy for interrupting transmission. The desired treatment coverage will be at least 90% of the eligible population (80% total population (therapeutic) coverage and 100% geographical coverage in all active transmission zones (‘foci’) in the country. When necessary and appropriate, targeted vector control or vector elimination, and use of any other effective intervention recommended by FMoH and its Elimination Expert Advisory Committee (EOEEAC), will be employed in specified

foci.The approach will be to tailor the interventions to the focus: unlike the former control approach, 'one size fits all' will not be the approach of the elimination program.

High treatment coverage especially based on 6-month intervals (semi-annual treatment) has the highest probability of the two MDA approaches of achieving elimination. Semi-annual treatment can be used to reduce transmission of *O. volvulus* L3s to the point that elimination of new infections occurs (Borsboom *et al*, 2003; Cupp *et al*, 1989).When the transmission was suppressed and no new infective larvae (L3s) were being introduced into the system (a 'closed system'), only 6.5 years (13 rounds) of semi-annual treatment were projected as needed to interrupt onchocerciasis transmission (Cupp & Cupp. 2005).The policy for semi-annual treatment in zones targeted for onchocerciasis elimination in Ethiopia is based on the successes of interruption of transmission observed after multiple dose per year community level ivermectin mass treatments with good coverage in the Americas, Sudan (Abu Hamad focus) and Uganda. Semi-annual treatments were launched in a number of woredas in Ethiopia in 2013 (including in Kafa and Sheka), after the declaration of the goal of elimination.

When required and where feasible other tools in specific instances, such as vector control/elimination or 3-4 weeks daily treatment with doxycycline, will be employed by the program in order to hasten transmission interruption.

Twice per year treatment will be indicated in any endemic area launching (after 2012) mass ivermectin drug administration for the first time (previously hypoendemic with transmission or previously unrecognized) or any annual treatment area that is not shown to be on a trajectory to be able to stop MDA by the target year of 2020. In the latter case, this would include areas with any one of the following: 1) skin snip positive rate among adults in any community is >2%; or, 2) any skin snip positive children<10 years of age in any community; or3) Ov16 rates in children <10 years exceed >0.1% (95% CI); or 4) PCR infectivity in flies exceeds >1/2000(95% CI); or 5) Seasonal Transmission Potential (as calculated by PoolScreen) exceeds 20 L3/person/year (95% CI).

3.3 Mapping

Delineation mapping has been conducted to determine the new treatment boundaries for every transmission zone. This delineation mapping added only wereda to the already known 178 districts of Ethiopia making the total endemic wereda in the country 179. It is now known how much does the endemicity extends from west to east by doing delineation mapping following APOC's crigging model. Looking into the ecological factors which might not favor the flies to breed and the lack of clinical evidence from hospitals, some experts challenge the importance of mapping the eastern part of the country. However, although it is speculated that ecological factors might not favor the transmission, an eastern extension of mapping is required, and the entire country must be assessed ecologically if in the future Ethiopia is to be successfully verified for onchocerciasis elimination by the World Health Organization. Mapping approaches should target for treatment any of the following:1) skin snip positive rate among adults in any community is >2%; or, 2) any skin-snip positive children<10 years of age in any community; or3) Ov16 rates in children <10 years exceed >0.1% (95% CI); or 4) PCR infectivity in flies exceeds >1/2000(95% CI); or 5) Seasonal Transmission Potential (as calculated by PoolScreen) exceeds 20 L3/person/year (95% CI).

3.4 Hypo-endemic areas

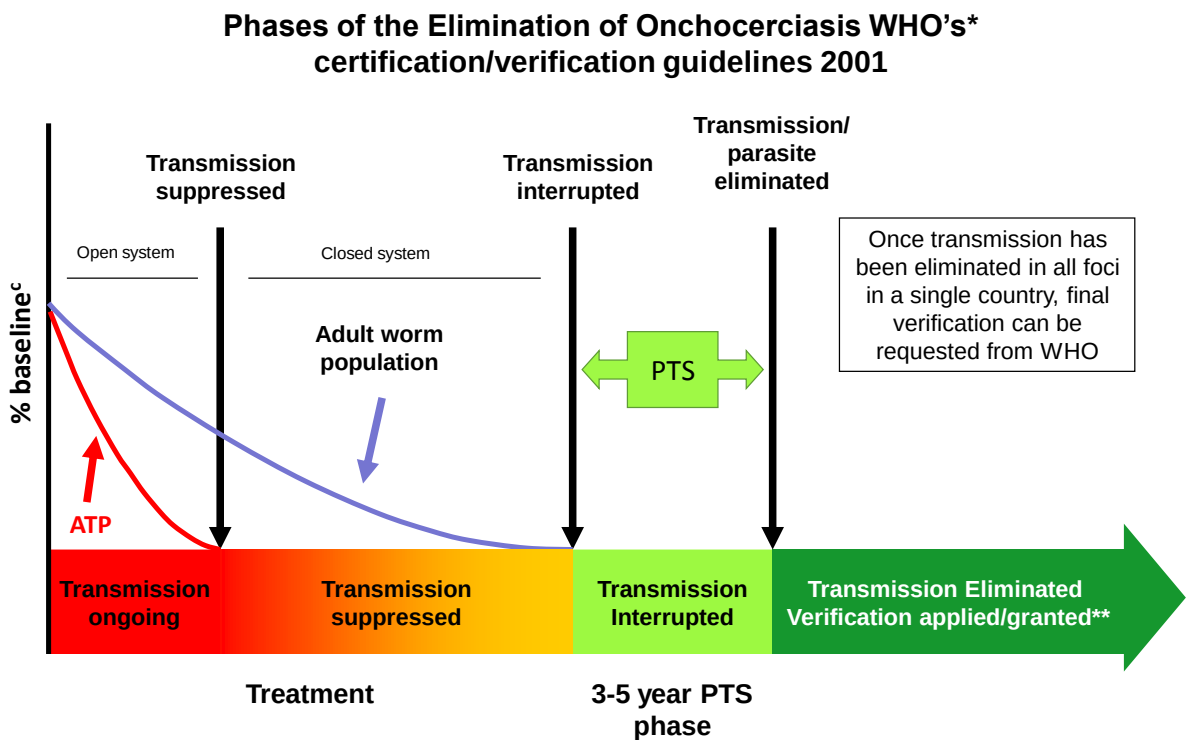
Hypo-endemic areas (with less than 20% nodule rate and/or less than 40% microfilaria prevalence) were not considered a priority under the former control strategy, and hence were not covered by MDA (Noma et al, 2004). The Ethiopian Onchocerciasis Elimination Strategy will now include all areas with active transmission, which includes the hypo-endemic areas in the MDA treatment scheme. In addition, the committee also will consider the following findings in treated or untreated areas as indicative of the need to start twice per year treatments: 1) skin-snip positive rate among adults in any community is >2%; or, 2) OV16 rates in adults exceeding 10%; or 3) any skin snip positive children<10 years of age in any community; or4) Ov16 rates in children <10 years exceed >0.1% (95% CI); or 5) PCR infectivity in flies exceeds >1/2000(95% CI); or 6) Seasonal Transmission Potential (as calculated by PoolScreen) exceeds 20 L3/person/year (95% CI).

3.5 Cross border issues

Onchocerciasis transmission zones appear to cross the border between the Sudan, the Republic of South Sudan (RSS), and Ethiopia.In addition, there is continuous movement of people who are possibly infected who cross into Ethiopia from Sudan and RSS. Onchocerciasis elimination requires cooperation between these countries. Therefore, in situations where these neighboring countries continue to have transmission, the program will work to address this cross border issue in exchanging information and prevent re-occurrence of disease. Support from other Ethiopian ministries (Defense, Foreign Affairs) and UN agencies (especially WHO) will be requests as deemed appropriate by the FMoH.

4. PHASES OF ONCHOCERCIASIS ELIMINATION PROCESS

The approach to elimination as defined in WHO Geneva guidelines is defined by four phases that are color coded (Figure 3):1) Red: Transmission ongoing (‘open system’); 2) Yellow: Transmission suppressed (‘closed system’); 3) Green: Transmission interrupted (MDA halted and post treatment surveillance ongoing); and 4) Dark Green: Transmission eliminated (post endemic surveillance, national dossier preparation).



*WHO Report, (2001). *Certification of elimination of human onchocerciasis: Criteria and procedures*. Following a WHO meeting on "Criteria for Certification of interruption of transmission/elimination of human onchocerciasis" (document WHO/CDS/CPE/CEE/2001.18a). Geneva, World Health Organization. This figure is unchanged in the WHO 2013 guidelines

Figure 3: Phases of Onchocerciasis Elimination, WHO, 2001

Dark Green	**Transmission Eliminated**
Light Green	Transmission Interrupted
Yellow	Transmission Suppressed
Red	**Transmission Ongoing**

Figure 4: The Epidemiologic "Oncho Flag" of Ethiopia

4.1. Transmission ongoing (pre-suppression, open transmission system) (red phase on graph and flag)

- The stage is characterized by positive PCR results in flies, mf prevalence, and Ov-16 antibody in adults and children.
- Activities during this phase could include delineation of transmission zones and establishing sentinel site(s) for in-depth epidemiological and entomological surveys to establish baseline prevalence data.

- Launch interventions—initiate regular once- or twice-per-year MDA with ivermectin for eligible populations targeting at least 90% coverage for an extended period of time.
- Initiate and maintain larviciding activities in the focus, if appropriate.
- Carry out regular community education and advocacy at all levels.

4.2. Transmission suppressed (yellow phase on graph and flag)

This stage is characterized by negative PCR in flies (<1 positive/2000 with 95% statistical confidence) and decreasing mf prevalence and serological (OV16 IgG4 antibody) positivity in children <0.1% (with 95% statistical confidence).If sufficient flies cannot be captured, Seasonal Transmission Potential (as calculated by PoolScreen) below 20 L3/person/year (with 95% statistical confidence).

- Do not stop treatment or transmission will likely recur.
- Carry out regular community education and advocacy at all levels.
- Conduct monitoring and evaluation activities.
- The intervention phase should end in the interruption of transmission, which is measured by entomologic, serologic and parasitologic results below accepted WHO thresholds.

4.3. Transmission interrupted/halting of ivermectin MDA phase (light green phase on graph and flag)

This stage is characterized by negative PCR in flies(<1 positive/2000 with 95% statistical confidence) and negative serological reactivity in children<10 years of age (<0.1% Ov16 reactivity with 95% statistical confidence).If sufficient flies cannot be captured, Seasonal Transmission Potential (as calculated by PoolScreen) below 20 L3/person/year (with 95% statistical confidence).

- Interventions are stopped after a careful review of that history and results of ‘Stop MDA’ evaluation results.
- In areas where lymphatic filariasis (LF) is co-endemic and ongoing treatment for this disease may continue after the decision to stop MDA for onchocerciasis has been taken. In this case the PTS period for onchocerciasis will be suspended pending completion of the LF Transmission Assessment Survey (TAS) that would allow all ivermectin MDA to be halted in the onchocerciasis focus.
- Initiate 3-5 years post treatment surveillance (PTS).

- After stopping MDA health education (information, education, communication) and consultation with affected communities and district authorities will continue.
- The program will maintain a supply of ivermectin tablets for clinical treatment of onchocerciasis cases as needed, which is based on frontline health units.

4.4. Transmission eliminated (dark green phase on graph and flag)

This begins at the end of the 3-5 year PTS period and is the confirmation of negative fly infections(<1 positive/2000 with 95% statistical confidence). If sufficient flies cannot be captured, Seasonal Transmission Potential (as calculated by PoolScreen) below 20 L3/person/year (with 95% statistical confidence).

- Supplemental epidemiological and serological surveys may be considered.
- Data for all 4 phases for the given transmission zone will be provided to the National Verification Committee (NVC) for archiving as a chapter in the national dossier to be ultimately provided to WHO after all transmission zones have reached this stage. The NVC or another independent team may wish to evaluate all the necessary data assembled by the country program.

4.5. Post-treatment surveillance

The beginning of the PTS period in a focus is usually (appropriately) a celebratory time. However, the celebrations are an opportunity for district authorities and the communities to be sensitized that halting interventionsis not the end of the program. There should be a strengthening of surveillance at all levels, including intensified community sensitization to the risk of resurgence of transmission.

The surveillance system should:

- Enhance sensitization about onchocerciasis in the national health care system including the communities by maintaining a high degree of public awareness of onchocerciasis and the need for immediate treatment with ivermectin.
- Maintain a community-based surveillance in all formerly onchocerciasis endemic foci supported by health extension workers (HEWs), community supervisors and HDA.
- Incorporate surveillance of other diseases (especially LF) into the surveillance system for onchocerciasis.

- Ensure periodic entomological surveys to monitor recrudescence or a vector re-invasion of previously free foci.
- Ensure monitoring and evaluation of community awareness about the disease and involvement in the implementation of intervention in the defined foci.
- Maintain proper documentation of all activities and reports which must be archived and stored properly at all levels (national, regional, zonal, district and community levels) for ease of verification by EOEEAC and International Verification Team (IVT).

5. MONITORING INDICES FOR THE PROGRAMME

5.1. Treatment coverage

Surveys following MDAs will be critical to monitor progress and increase coverage. Treatment coverage will be reported annually or semi-annually and have the following indices:

- Geographic coverage:% communities treated of total number communities in a transmission zone (focus).
- Eligible population coverage: % eligible persons treated of total number eligible persons targeted in the focus.
- Therapeutic coverage: % eligible persons treated of total population in the focus.
- UTG coverage: % eligible persons treated of total eligible persons to be treated with 100% geographic coverage is reached in the focus.
- UTG(2) coverage: % eligible persons treated in the entire year of total eligible persons to be treated multiplied by 2 based on the UTG to be treated with 100% geographic coverage is reached in the focus.
- Surveyed coverage: survey questionnaire of coverage based on a statistically appropriate sample, with 95% confidence interval.

5.2. Epidemiological (parasitological) assessments

These surveys are conducted in a way to determine prevalence breakdown by age and gender.

- Skin snip surveys (microscopy and PCR based, see Standard Operating Procedures in Appendix 3). PCR testing will take place at the onchocerciasis molecular laboratory that

has been established at the Ethiopian Public Health Institute (EPHI) with assistance from The Carter Center.

5.3. Serological assessment

At least 2000 children under ten years of age sampled throughout the focus will be tested with OV16 IgG4 antibody test will serve as a the major assessment criteria to validate interruption of transmission and make the stop MDA decision. The analysis should allow stratification by age and location. The overall rate should be <0.1% infection with 95% statistical confidence.

In situations where a small number of sero-positive children drive the overall seropositivity rate slightly above 0.1%, skin snips on those positive children will be evaluated by PCR for the presence of *O. volvulus* microfilariae.If serologically positive children were found negative by PCR testing of skin snips they are considered to have been exposed but not infected, and thereby negative for the purpose of calculating the 0.1% threshold.

Serological testing will ideally take place at the onchocerciasis molecular lab that has been established at the Ethiopian Public Health Institute (EPHI) with assistance from The Carter Center and its reference laboratory at the University of South Florida, USA.

5.4. Entomological assessment

a. Entomological evaluation by PCR technique for *Simulium* spp.

- Pool screen PCR using *O. volvulus* –specific assays, or other valid methods. The prevalence of flies carrying infective larvae (L3) should be below 0.05% (<1/2000) (assuming a parity rate of 50%) with 95% statistical confidence. To confirm that the transmission is interrupted or has in effect been eliminated the following results should be obtained: sufficient flies must be tested to insure that the upper boundary of the 95% confidence interval for infective flies is below 0.05%. Obtaining this level of statistical confidence requires at least 4000 flies from a community be tested.
- Dissection of black flies to determine infection rates is not recommended given that *O. ochengi* is extremely difficult to distinguish microscopically from *O. volvulus*.
- Seasonal or Annual Transmission Potentials (STP and ATP respectively) should be calculated using the PoolScreen algorithm.

PCR testing will take place at the onchocerciasis molecular lab that has been established at the Ethiopian Public Health Institute (EPHI) with assistance from The Carter Center.

b. Entomological evaluation for *Simulium neavei* and *Simulium ethiopiense*

Entomological evaluations will be carried out in the same way (as described in section 5.4.a above) for all foci no matter whether vectors are *S. damnosum* s.l., *S. neavei* group (=*S. ethiopiense*) or a mixture of both sorts of vectors. However, in some *neavei*-foci (in other countries) biting rates can be very low and it can be difficult to obtain sufficient vectors for pool-screening. Similarly, if vector control is used to support CDTI there may be a shortage of vectors for pool-screening. In both circumstances pool-screening of vectors will continue to the mainstay of entomological evaluation (albeit with sub-optimal sample sizes), but if vector control against *S. ethiopiense* is introduced crab examination, additional fly traps per site, and additional sites will also be used for entomological evaluation.

In *S. neavei-/S. ethiopiense*-dominated foci under vector control and/or vector elimination, crabs and fly catches will be used. The lack of positive crabs for larvae/pupae and the absence of adult flies in human attractant fly catches conducted in a defined focus in a series of surveys over a period of 3 years are indicative of interruption of transmission (Garms, *et.al,* 2009).Under such a situation, there will be no flies to subject to PCR pool screen and serologic evaluation in children, in conjunction with an absence of flies, will be valuable in confirming interruption of transmission.The FMoH, considering the recommendations of the EOEEAC, will need to evaluate individual foci/transmission zones on a case by case basis, using all best available data.

6. ETHIOPIA ONCHOCERCIASIS COMMITTEES ENGAGED IN THE ELIMINATION PROCESS

The preparation of Ethiopia for verification of onchocerciasis elimination is a multi-faceted process involving many stakeholders.It is envisaged that the development partners, and especially the WHO, will continue to provide technical support in the form of consultants from time to time to not only strengthen the national capacity but also steer the process of preparedness for verification.

6.1 Ethiopia Onchocerciasis Elimination Expert Advisory Committee (EOEEAC)

EOEEAC will be formed with the objective of providing technical advice to the FMoH on onchocerciasis elimination. This advisory committee is composed of national and international experts, as well as ministry of health staff from all the endemic regions. The committee will review annual progress of the national elimination effort and update the Ethiopia 'onchocerciasis flag', which will be a visual table color coded by focus according to the four stages of elimination depicted in the WHO guidelines. Further, the committee will recommend altering interventions or halting interventions in a particular focus.It is the responsibility of FMoH to decide on altering interventions or halting interventions and to ensure proper communication with the respective regions, zones, and/or district authorities and affected communities about the decisions made.

6.2 National Verification Committee

The National Verification Committee (NVC) will be constituted at the appropriate time by the FMoH to develop the country dossier to be submitted to WHO for formal verification of elimination of onchocerciasis from the country. The NVC will be responsible for the preparation activities leading to verification that must be carefully planned and implemented to ensure that all items and data needed will be in place by the time for the international verification team (IVT) from WHO is invited into the country. It will be the committee to liaise with the WHO IVT.

Since the attainment of verification level will be achieved at different times in the various onchocerciasis endemic foci, there will be two kinds of reporting. Firstly, detailed reports will be compiled on every focus that has attained verification status as verified by the NVC. Secondly, when all the different foci have attained verification status, the NVC will compile a final report for the whole country.These reports may vary but must be factual, evidence based, and the contents of which should include the following:

- Historical overview of onchocerciasis in the foci (transmission zones) in the country and details about the implementation of the elimination campaign in each focus, including challenges and lessons learnt during the campaign. Publication of the experience in each focus in the peer-reviewed literature is strongly encouraged to facilitate the verification process.

- Overview of the administrative system and health care infrastructure and delivery system as well as the capacity to detect and manage cases should such occur in future.
- A critical review of the threat of recrudescence of the disease in previously freed foci/CDTI zones from the neighboring foci or countries.
- An evaluation of the effectiveness of the routine disease reporting system, and how the system could capture information on onchocerciasis should such cases occur in the future and how feedback information could be passed.
- The report should use maps and graphs to illustrate changes in the disease trends and distributions as well as for health / administrative infrastructures in the country.
- The report should present coherent recommendations on post-verification interventions to check recrudescence or re-introduction of the disease in the freed foci or country as a whole. In this section the knowledge about the absence of onchocerciasis outside of traditionally recognized endemic areas should be given.

When the country report has been compiled and accepted by NVC, the members of the committee including the secretariat must sign it before submitting to FMoH for adoption and subsequent submission to the WHO country office. Based on the country report and the recommendations of the NVC, the FMoH will sign a declaration to the effect that indeed onchocerciasis has been eliminated in Ethiopia and will invite the International Verification Team (IVT) to come to Ethiopia to verify that Ethiopia is free of onchocerciasis.

The Federal Ministry of Health and EPHI will be responsible for storing all data generated by the program, and providing the NVC with secretariat support.

6.3 International Verification Team

The International Verification Team (IVT) will be constituted by WHO Geneva Headquarters in response to an official request by the Government of Ethiopia for formal verification of elimination of onchocerciasis. The ICT verifies submitted reports through field visits, interviews national program staff and members of the various committees (EOEEAC and NVC), and reviews documents at all levels. It then will issue a report to WHO recommending verification or not. If a positive report is accepted by the Director General (DG) of WHO, an official letter signed by the DG indicating verification is sent to the minister of health of Ethiopia.

Even after such verification, the FMoH will remain committed to ensuring that onchocerciasis does not reestablish itself once officially eliminated in Ethiopia.

7. CONCLUSION

The permanent interruption of transmission of onchocerciasis in Ethiopia by 2020 and obtaining WHO official verification of this accomplishment soon thereafter, is the goal of the new elimination program. The elimination process described in this document is based on the WHO Geneva guidelines that outline the process required to accomplish this goal.

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APPENDIX 1: FACTS SHEET ON ONCHOCERCIASIS

Key facts

- Onchocerciasis, commonly known as "river blindness" is caused by the parasitic worm *Onchocerca volvulus*.
- It is transmitted to humans through exposure to repeated bites of infected blackflies of the genus *Simulium*.
- Symptoms include severe itching, disfiguring skin conditions and visual impairment, including permanent blindness.
- More than 99% of infected people infected live in 31 African countries; the disease also exists in some foci in Latin America and Yemen.
- Community-directed treatment with ivermectin is the core strategy to eliminate onchocerciasis in Africa; in the Americas the strategy is semi-annual large-scale treatment with ivermectin.
- In 2013 and after a verification process, WHO declared Colombia free of onchocerciasis.

Onchocerciasis – or "river blindness" – is a parasitic disease caused by the filarial worm *Onchocerca volvulus* transmitted by repeated bites of infected blackflies (*Simulium* spp.). These blackflies breed in fast-flowing rivers and streams, mostly in remote villages located near fertile land where people rely on agriculture.

In the human body, the adult worms produce embryonic larvae (microfilariae) that migrate to the skin, eyes and other organs. When a female blackfly bites an infected person during a blood meal, it also ingests microfilariae which develop further in the blackfly and are then transmitted to the next human host during subsequent bites.

Signs and symptoms

Onchocerciasis is an eye and skin disease. Symptoms are caused by the microfilariae, which move around the human body in the subcutaneous tissue and induce intense inflammatory responses, especially when they die. Infected people may show symptoms such as severe itching and various skin lesions. In most cases, nodules develop under the skin. Some infected people develop eye lesions that can lead to visual impairment and permanent blindness.

Onchocerciasis occurs mainly in tropical areas. More than 99% of infected people live in 31 countries in sub-Saharan Africa: Angola, Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Republic of Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Ethiopia, Gabon, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Sudan, Sudan, Togo, Uganda, United Republic of Tanzania. It has also been introduced in Yemen.

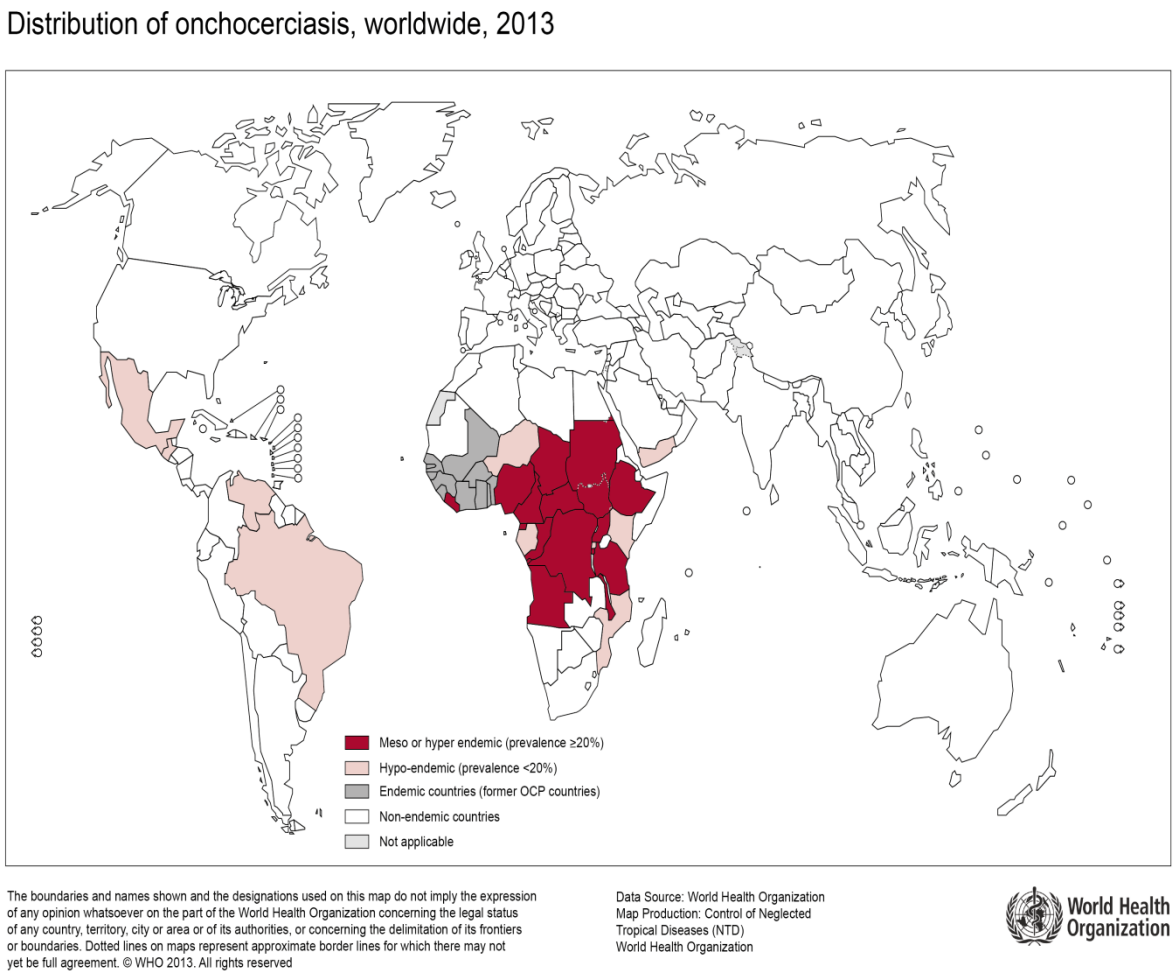

Figure 5: Distribution of Onchocerciasis Worldwide, 2013

Onchocerciasis is also found in 11 foci scattered in 4 countries in Latin America: Brazil, Guatemala, Mexico and Venezuela (Bolivarian Republic of).

Prevention, control and elimination programmes

There is no vaccine or medication to prevent infection with *O. volvulus.*

Between 1974 and 2002, onchocerciasis was brought under control in West Africa through the work of the Onchocerciasis Control Programme (OCP), using mainly the spray of insecticides

against blackfly larvae (vector control) by helicopters and airplanes. This was supplemented by large-scale distribution of ivermectin since 1989.

The OCP relieved 40 million people from infection, prevented blindness in 600 000 people, and ensured that 18 million children were born free from the threat of the disease and blindness. In addition, 25 million hectares of abandoned arable land were reclaimed for settlement and agricultural production, capable of feeding 17 million people annually.

In 1995, the African Programme for Onchocerciasis Control (APOC) was launched with the objective of controlling onchocerciasis in the remaining endemic countries in Africa. Its main strategy has been the establishment of self-sustaining community-directed treatment with ivermectin, and, where appropriate, vector control with environmentally-safe methods.

In 2010, nearly 76 million ivermectin treatments were distributed in 16 APOC countries where the strategy of community-directed treatment with ivermectin (CDTI) was being implemented. At least 15 million additional people need to be reached in the next few years as the programme has now shifted from control to elimination.

The Onchocerciasis Elimination Program of the Americas (OEPA) began in 1992 with the objective of eliminating ocular morbidity and transmission throughout the Americas by 2012 through biannual large-scale treatment with ivermectin. All 13 foci in this region achieved coverage of more than 85% in 2006, and transmission was interrupted in 10 out 13 by the end of 2011.

Following successful large-scale treatment of populations in affected areas with the support of international partners, Colombia and Ecuador were able to stop transmission of the disease in 2007 and 2009 respectively. Mexico and Guatemala were also able to stop transmission in 2011. Elimination efforts are now focused on the Yanomami people living in Brazil and Venezuela.

On 5 April 2013, the Director-General of WHO issued an official letter confirming that Colombia has achieved elimination of onchocerciasis. The President of Colombia publicly announced this WHO verification in a ceremony held in Bogota on 29 July 2013. Colombia has since become the first country in the world to be verified and declared free of onchocerciasis by WHO.

Treatment

WHO recommends treating onchocerciasis with ivermectin at least once yearly for about 10 to 15 years. Where *O. volvulus* co-exists with *Loa loa*, another parasitic filarial worm that is endemic in Cameroon, the Central African Republic, the Congo, the Democratic Republic of the Congo, Nigeria and South Sudan, it is recommended to follow the Mectizan Expert Committee (MEC)/APOC recommendations for the management of severe adverse events that may occur.

WHO response

WHO is the executing agency of APOC. The WHO Regional Office for Africa supervises APOC's management, while WHO headquarters provides administrative, technical and operational research support. Through the OEPA partnership, WHO collaborates with endemic countries and international partners.

WHO is currently facilitating the launch of an elimination programme in Yemen in collaboration with the Ministry of Health, the World Bank and other international partners.

APPENDIX 2: DEFINITIONS RELEVANT TO ONCHOCERCIASIS ELIMINATION

An endemic onchocerciasis focus: is an area within a country where a local cycle of *Onchocerca volvulus* transmission is maintained and is giving rise to autochthonous infections. In terms of population biology of the parasite, this is an area where the basic reproduction ratio (Ro) is 1 or greater. Endemicity is stable where the incidence of the infection shows little or no trend to increase or decrease over time. An endemic focus is sometimes referred to as a Transmission Zone to emphasize that transmission is more or less independent of immigration of parasites carried by flies or by humans.

An onchocerciasis case: is defined as an individual with evidence of current infection with *Onchocerca volvulus*.

Annual biting rate: is the number of flies that will bite a person positioned at the study site in a year.

Annual Transmission Potential (ATP):is a value calculated as the product of the annual biting density, the proportion of flies with infective-stage *O. volvulus* larvae, and the mean number of infective larvae per infective fly. The value refers to the approximate number of infective larvae any one individual may be exposed to in a years' time.

Case definition: In an endemic area a person presenting with fibrous nodules in subcutaneous tissue. Laboratory confirmation is the presence of microfilaria in skin snips taken from the iliac crest, the presence of adult worms in the excised nodules and presence of ocular manifestations, such as slit lamp observations of microfilaria in the cornea, the anterior chamber or vitreous body.

Verification: a country will be eligible for verification as being in post endemic phase after successfully completing a 3-5 year pre-verification period in all its foci.

Elimination: (literally "casting out over the threshold) of the parasite population from a defined geographical area means the sustained absence of transmission until the adult parasite population within that area has died out naturally or has been exterminated or sterilized by some other intervention. This should occur within 15 years after interruption of transmission or in as short a time as 6.5 years with administration of semi-annual ivermectin. When elimination of the parasite is verified, the endemic area moves into the "post endemic" phase.

Endemic: when onchocerciasis morbidity, transmission and infection are present.

Endemicity: is the permanent presence of the disease or pathogenic agent in a given region. Its level is determined according to the presence of the disease or pathogen, i.e. the percentage of diseased persons or carriers in a given population.

Geographical coverage: meaning percentage of endemic communities receiving ivermectin. The requirement being that all endemic (100%) communities be identified and receive a regular mass distribution of ivermectin.

Incidence: is the rate at which new cases arise in a population within a defined interval.

Interruption of transmission: means the permanent interruption of transmission in a clearly defined area (focus) after all the adult worms in the human population in that area have either died out from old age or been exterminated by some other intervention. This should occur within 6.5 years of the establishment of sustained interruption of infectivity in areas receiving semi-annual MDA with ivermectin.

MDA: Mass drug administration.

Morbidity: is defined as the presence of a disease manifestation caused by onchocerciasis.

Post Treatment surveillance (PTS):refers to that period (3-5 years) following termination of treatment during which ongoing surveillance is conducted to document that interruption of transmission has occurred and there is no recrudescence of transmission.

Post-endemic: When a focus or country with a past history of endemic onchocerciasis is officially certified as having successfully completed a 3-5 year pre-verification period of interrupted transmission in all its previously endemic onchocerciasis foci.

Pre-verification period: is the period following interruption of transmission, during which surveillance is carried out to verify that interruption of transmission has been sustained after ceasing control interventions. This period lasts for 3-5 years during which no intervention is carried out.

Prevalence: is the proportion of the host population infected at a particular point in time.

Program coverage: meaning percentage of the eligible population treated. The targetis treatingat least 90% of the eligible population in each community ineach round.

Seasonal Transmission Potential (STP):is a value calculated as the product of the seasonal biting density, the proportion of flies with infective-stage larvae, and the mean number of infective larvae per infective fly. The STP may be equal to or slightly less than the ATP.

Semi-annual treatment: Six monthly mass drug administration.

Sentinel communities: are preselected hyper-endemic communities where in-depth epidemiological and entomological evaluations take place at regular intervals; first before treatment starts, then again after two years and finally at 4-year intervals thereafter. The evaluations include parasitological (mf and nodules), ophthalmological, and entomological indicators. It should be noted that the use of sentinel communities in this way has two disadvantages. First, the community populations may become tired of these repeated examinations and refuse to cooperate. Second, it will soon become known by those working in

the programs, which are the designated sentinel communities, and they may reserve their best efforts for these communities at the expense of others. A possible way around this difficulty is to have a larger number of potential sentinel communities and just before each round of examinations to pick at random a smaller number of them that will be examined.

APPENDIX 3: STANDARD OPERATING PROCEDURES FORCOLLECTING SKIN SNIP FOR DIAGNOSIS OF *ONCHOCERCAVOLVULUS* PARASITE

Site Selection based on: high baseline prevalence data, proximity to the main breeding sites, isolated and representative of the transmission zones.

Preparation for Skin snip exercise:

Community assessment for onchocerciasis infection by skin snip should be well planned and communities mobilized in advanced of planned survey in order to enlist their cooperation and participation. Rainy season should be avoided since it may not only make it difficult to access some villages but people may be busy in their fields contributing to poor compliance.

Procedure of conducting skin snip:

Before taking a skin snip, ensure that there is privacy for participants. The selected site for skin snip is usually left and right iliac crest; ensure that this is swabbed with an alcohol pad. Remove a small bloodless fragment of skin from the iliac crest area, using a punch (Walser punch). The skin biopsy is placed in 0.1 normal saline in a microtitre plate. It is allowed to incubate for 24 hours (WHO, 1995), and examined under a compound microscope for microfilariae. In the event of a positive test, one observes the free microfilaria under the binocular microscope. The number of microfilaria is enumerated in each skin snip and from that prevalence, density, and Community microfilariae load are calculated (WHO/APOC, 2002).

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APPENDIX 4: STANDARD OPERATING PROCEDURES FOR HUMAN BAITED CATCHES OF *SIMULIUM* ADULT FLIES

Choice of catching point

Site selection as catching points is critical and should be based on the following criteria and should preferably be situated close to sentinel communities identified for periodic epidemiological studies. The criteria for selection include:

- The catching point should be located in a zone where the conditions of transmission of infection are met: presence of man, parasite and vector.
- Catching points should be representative of the transmission foci
- It should be near a significant and productive breeding site.
- It should be in a shade and sheltered or protected.
- It must be accessible all the year round.
- It should not be a place of human gathering, in order to avoid at the same time dilution and a drop in the vigilance of the people engaged in trapping the flies

Catches on humans (human bait)

The catches made on humans make it possible to know the density of the population of biting females of *S. neavei or S. damnosum s.l.* , in a given place and period, and to estimate it in number of bites/man/day. The use of man as bait is common for catching many hematophagous insects.

Collector teams

Each catching team is made up of two people who carry out catches of *Simulium* for one hour in turns. It is known that *S. neavei/ S. damnosum s.l.* bite preferably the lower limbs. Taking into account this low location of the bites of females, only the legs of the catcher are exposed. The black flies that alight are immediately covered, then trapped in catching tubes, on a rate of one fly per tube. The black flies thus caught are, in theory, meant for a study of their infection by *Onchocerca volvulus*. Thus it is absolutely necessary that the females, which come to bite, be caught before they start their process of biting, in order to avoid loss of parasites, and the potential infection of the catcher.

On the other hand, when one wants to collect engorged females (in particular on *Onchocerca* patients), any female that alights on the catcher and starts a blood meal is immediately covered by a tube. When the *Simulium* is completely gorged, it is detached spontaneously from the

skin and is collected into a tube. Sampling is usually carried out on the basis of 7:00-18:00 hours continuous catching day, which is the activity period of the *Simulium.* The results are recorded on a catching form (WHO/APOC, 2002).

Reference

WHO/APOC (2002).*Training module for national Entomologist in the Management and supervision of entomological activities in Onchocerciasis control*

APPENDIX 5: STANDARD OPERATING PROCEDURES FOR CRAB CATCHING AND EXAMINATION IN *SIMULIUM NEAVEI*GROUP

Where there are no pre-control data confirming the species of vector it has to be assumed that either vector may be involved. Therefore, surveys must be directed at both *S. damnosum s.l* and *S. neavei*, until evidence confirms which vector is involved.Where the vector is a member of the *S. damnosum*complex surveys should be followed as outlined in Appendices 4 and 6 of this document (WHO 2001).

Where the vector is *S. neavei* entomological surveys should primarily employ trapping and examination of crabs for infestation with the immature stages of *S. neavei*. This is more sensitive and less costly than human landing fly catches. It has no adverse ecological consequences, as the crabs are returned unharmed to their breeding rivers.Crab trapping must be carried out at least twice annually, at each site, for three years, a minimum of two traps to be used on each occasion. This is sufficient to establish the presence or absence of crabs and infestation.

It is not possible to specify the number of necessary trapping sites, as this will depend on the size of the focus and the complexity of the hydrological network. Coverage must be comprehensive, which means at least 3 sites per medium-sized river (one each in upper, middle and lower reaches). Such sites should not be more than 10 km apart. Small streams may be monitored by single trapping sites. Where a survey shows the presence of infested crabs, detailed investigations (including human landing captures) may be carried out.

Crab catching and examinations are always done to determine the infestation of freshwater crabs. The immature stages (larvae and pupa) of all vector species live attached to fresh water crabs (*P. aloysiisabaudiae, P. niloticus, P. loveni)* in phoretic association. Phoretic rates of more than 50% have been reported in a number of foci in Uganda (Barnley, 1975). Locally made basket traps are used in crab catches using the following procedures:

Camouflaged baited traps are placed in appropriate sites along a river course; most preferably stony with not very fast flowing water should be selected for placing the trap. Traps are always placed with the side opening facing towards the current, to allow water to carry the scent of the bait some distance away from the trap. The duration of trapping is one hour (Garms, *et al*, 2009), but in places where crabs are few it can be left overnight. Later, the traps

are removed and are emptied into a container with clean water for identification, carapace size measurement with vernier calipers and examined for larvae or pupae of *S. neavei.* The number of immature stages are counted and then recorded on a crab form. The GPS locations of sites where catches are conducted are always recorded.

There is clear evidence that *S. neavei* takes 18-36 months to recover from local elimination. Therefore, it is recommended that the 3 year post treatment surveys should only begin 1 year after the cessation of treatments.

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APPENDIX 6: FIELD PROTOCOL FOR THE DEPLOYMENT OF ESPERANZA WINDOW TRAP FOR THE COLLECTION OF *SIMULIUM DAMNOSUMS.L* IN ETHIOPIA

1. FIELD MATERIALS

Yeast, white sugar, water, polymer trap 1m X 1m (black striped with blue at the middle), aluminum frame, pegs, tangle foot, brush, gloves, ropes, scissors, rope, copper wire, 5Litre plastic bottle, tubing, forceps, bijou bottles, white mineral spirit (or kerosene), 80% alcohol, human scent lure, field recording book, recording form, biro.

2. CONSTRUCTION OF THE TRAP (For a trap)

-Paint the madabara (local polymer) in black striped with blue color at the middle. Allow the paint to dry completely before being transported to the field.

- Fix the polymer into an aluminum frame. The interior must be 1m by 1m.

3. PRE-FIELD PREPARATION

a. Preparation of organic Carbon-dioxide

- weigh 60g of yeast
- weigh 500g of sugar
- 2 Litres of clean water

Mix all in a 5 Litre plastic bottle.

Note: The organic carbon dioxide should be prepared 2 hours before the commencement of the trapping and should only be used for two days.

FIELD SET-UP OF THE TRAPS (Conditions: Set the traps perpendicular to the identified breeding sites).

Steps in setting up the traps

1. Make a few centimeter dig to erect the two pegs (1 meter apart) at the selected trapping site
2. Hook the trap firmly to the iron rods/pegs at both ends using copper wire or rope (ensure that the trap is about 10 cm above the ground level).
3. Sandwich/coat the two surfaces of the trap with tangle foot (glue)
4. Place the tubing connected to the plastic with yeast-sugar mix to release carbon dioxide to the trap

5. The tubing should be connected to the side of the trap about 30 cm above ground level
6. Place the sweat lure at about 30 cm above ground
7. Studies have shown that baiting the trap with sweat-soaked clothing worn by a villager (in addition to sweat lure and Carbon dioxide) can enhance catches
8. Ensure that the traps are placed around the breeding sites, and undisturbed places

Note: When tangle foot is diluted with kerosene, the trap should be allowed to stand for at least 24 hours before the commencement of trapping to allow the kerosene to evaporate completely.

4. RECOVERY OF BLACK FLIES FROM THE TRAP
    1. Recover *Simulium damnosum* flies trapped once daily (5:00pm) using forceps
    2. Put the black flies in plastic container with white mineral sprite or kerosene
    3. Allow it to stay for few minutes
    4. Count the number of the flies and transfer them to the bottle containing 80% alcohol
    5. Remove other trapped insects from the trap to keep it clean every recovering hour or before the commencement of daily trapping.
    6. There may be need to confirm the flies under microscope

APPENDIX 7:STANDARD OPERATING PROCEDURES FOR OV-16 ELISA ASSAY

Introduction

Antibodies specific for *Onchocerca volvulus* can be found in the blood, but may not indicate active infection. Assays for *O. volvulus* antibodies include the Ov16 card test (to detect IgG4 targeting Ov16 antigen), ELISA to detect antibodies against cocktails of recombinant antigens (e.g., Ov20 and Ov33 or Ov7, Ov11 and Ov16), and dot blot assay using native adult parasite antigens. These are currently available only in a research setting and are not approved for clinical diagnosis. Ov16 ELISA is used in the Onchocerciasis Elimination Program in the Americas to determine if transmission has been eliminated. The technique is a very sensitive tool which detects the lowest levels of onchocerciasis transmission.

Purpose

The dry blood spots are eluted and examined to determine the exposure of children to infection in onchocerciasis endemic areas where the disease elimination has been launched.The technique is a sensitive epidemiological tool for monitoring and evaluation of *O. volvulus* elimination program. The presence of antibodies specific for the antigen is an indicator of exposure.

Specimen required

Finger prick blood collected on Whatman filter paper #2. The dry filter paper sample must be stored at -20 degrees Celsius in the presence of a dessicant.

Materials and reagents required

Sample Collection

Cotton wool

Disinfectant solution

Sterile single use lancets

5 x 5cm Whatman filter paper#2

Plastic ziplock envelopes

Dessicant

Cool box and ice packs

Pencils

Drying rack

Sharps disposal box

Sample Elution

Punching machine

6mm punch of the sample spot

96 well plastic ELISA plates

5% Bovine serum albumin

Multi-channel pipette (P -200)

P-200 tips

Elution is done at 2-8 degrees Celsius overnight

A. Reagents

1. PBS 10X: (store at room temperature)

- NaCl 80 g
- KCl 2.0 g
- KH_2PO_4 2.4g
- Na_2HPO_4 14.4 g

Final volume 1L, pH 7.0-7.4

2. PBST: (store at room temperature)

PBS 1X

Tween 20 0.05%

3. PBST-BSA (store at 4°C)

1x PBST, Bovine serum albumin 5% (W/V)

4. Coating Buffer (store at 4°C)

0.1 M $NaHCO_3$ (pH 9.6)

5. 1M $MgCl_2$

6. Substrate buffer (store at 4°C in the dark)

Diethanolamine 10% (V/V)

$MgCl_2$ 3 mM

Adjust pH to 9.8

7.1 mg/ml p-Nitrophenyl phostate (pNPP) solution (Sigma N-9389)

8. Anti-human IgG4 antibody conjugated to biotin (Zymed 05-3840)

9. Streptavidin-AP conjugate (Invitrogen 19542018)

10. Ov16 GST antigen

11. Positive Control Sera:

Constructed of a pool of at least 20 serum samples from patently infected people.Prepare twofold serial dilutions starting with 1/20, in PBST-BSA.

12.Immunolon 2HB flat bottomed plates (Thermo Scientific)

B. Procedure

1. **Create a map:** Use the map below. The samples are run in duplicate as indicated.

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1:20	STD 1:20	STD 1:5120	STD 1:5120	S5	S9	S13	S17	S21	S25	S29	S33
B	STD 1:40	STD 1:40	STD 1:10240	STD 1:10240	S5	S9	S13	S17	S21	S25	S29	S33
C	STD 1:80	STD 1:80	B	B	S6	S10	S14	S18	S22	S26	S30	S34
D	STD 1:160	STD 1:160	B	B	S6	S10	S14	S18	S22	S26	S30	S34
E	STD 1:320	STD 1:320	S1	S3	S7	S11	S15	S19	S23	S27	S31	S35
F	STD 1:640	STD 1:640	S1	S3	S7	S11	S15	S19	S23	S27	S31	S35
G	STD 1:1280	STD 1:1280	S2	S4	S8	S12	S16	S20	S24	S28	S32	S36
H	STD 1:2560	STD 1:2560	S2	S4	S8	S12	S16	S20	S24	S28	S32	S36

STD = positive control pool dilutions

B = blank wells

S = sample wells

2. **Elution from paper filter samples:** Punch out duplicate spots from the dried blood samples collected on the filter papers using a standard 6mm paper punch. Using your map, place the duplicate punches of the blood spots into the sample wells. Add 200µl of PBST-BSA to each sample. Push the punches to the bottom of the well and then mix 10 times by pipetting. Cover the plates with a plate sealer and incubate them at 4°C overnight. Store the eluted serum samples at -20^{o}C.

3. **Coating plate with antigen**: Dilute Ov16-GST antigen to 2.0 µg/ml in coating buffer. Add 100 µl to each well. Place the plate in a ziplock bag and incubate it overnight at 4°C.

4. **Washing plates**: Wash 4 times with PBST, using a wash bottle. Do not dry between washes. But dry the plate after last wash.

5. **Blocking plates:** Add 100 µl of PBST-BSA, place the plate in a ziplock bag and incubate it at 4°C for 1 hour.

6. **Preparation of standard and controls:** During the incubation of step 5 prepare 10 serial two fold dilutions of the positive control pool, beginning with a 1:20 dilution.

7. **Empty plate**: After the incubation of step 5, empty the PBST-BSA in the sink and dry the plate. Do not wash.

8. **Adding samples**: Use your map, add 50µl of each serum sample (positive control dilution, blanks and eluted samples) to the corresponding wells on the plate map. Place the plate in a ziplock bag and incubate it at room temperature for 2 hours.

9. **Washing plates**: Wash 4 times with PBST, using a wash bottle. Dry the plate after the first wash, then carry out the remaining three washes without drying the plate between the wash steps. Dry the plate after the 4^{th} wash.

10. **Add conjugate**: 10 minutes before the time is up for the last incubation, prepare the conjugate. Dilute the anti-human IgG_4 antibody conjugated to biotin 1:1000 in PBST. Add 50µl of the diluted conjugate to all wells. Place the plate in a ziplock bag and incubate it at room temperature for 1 hour.

11. **Washing plates:** Wash 4 times with PBST, using a wash bottle. Do not dry between washes. But dry the plate after last wash.

12. **Add Streptavidin-AP**: 10 minutes before the time is up for the last incubation, prepare the streptavidin-AP. Dilute streptavidin-AP 1:2000 in PBST. Add 50µl to all wells cover the plate and incubate it at room temperature for 1 hour.

13. **Washing plates:** Wash 4 times with PBST, using a wash bottle. Do not dry between washes. But dry the plate after last wash.

14. Substrate: 15 min before the incubation period is over switch on the ELISA plate reader. Prepare PNPP solution with the substrate buffer by dissolving 1 tablet in 5ml of the buffer provided. Add 50µl to each well. If your reader reads the whole plate at once, then read the plate until the 1/20 standard is around OD 1.5. If your reader reads well by well, then read only the two wells of the 1/20 standard every minute, and shake the plate between readings. When they are around 0.9 OD, start reading more frequently. When you reach OD 1.1, stop the reaction by adding 25µl of 3M NaOH to each well. Shake the plates to stop the reaction. Incubate the plate for 5 minutes at room temperature and read it at 405nm.

C. Quality assurance

All the ELISAs should be analyzed using the following criteria; if the ELISA run does not meet these criteria it should be repeated.

The standard curve dilutions are assigned the following arbitrary units:

Dilution	Units
1:20	2560
1:40	1280
1:80	640
1:160	320
1:320	160
1:640	80
1:1280	40
1:2560	20
1:5120	10
1:10,240	5

A plot of OD versus units should be linear in the range of 20-160 units, with an r^2 value for the line of >0.95.Duplicate wells in the standard curve should be within 10% of one another.The negative control wells should exhibit an OD value corresponding to <20 units.

D. Analysis of Results

The cutoff is set at 40 arbitrary units. Any sample for which both duplicate samples give a reading at or above this point is considered putatively positive.If the sample is putatively

positive, repeat the assay.Samples giving an OD value greater than 40 units in the confirmatory assay are confirmed positives.

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APPENDIX 8: STANDARD OPERATING PROCEDURES FOR O-150 POOL SCREEN PCR FOR SIMULIUM FLIES

Introduction

In Ethiopia Onchocerciasis is mainly transmitted by *S. damnosum and S. neavei* to some extent. The third stage infective larvae are found in the head of the vector.The transmission rate of the parasites by the vector can be determined by molecular methods. O150 PCR is a molecular epidemiology technique which detects a tandemly repeated 150 base pair DNA segment of the parasite in the vector.

Purpose

For monitoring *O. volvulus* transmission by *Simulium* vector flies.

Specimen Required

Adult *Simulium* vector flies caught using the human bait method.

Materials and reagents required

Materials for DNA extraction and purification

1.5 ml Snap cups, 95% Ethanol, pipette tips, pasture pipettes, NaCl, Tris HCl, EDTA, SDS, Carrier DNA solution, plastic homogenizers, protease solution, heating blocks, thermometer, DTT, freezer, 96 well filter plates, , Ethanol wash, Elisa plates, , distilled water

Materials for DNA amplification

PCR water, PCR tubes, DNTPs, taq polymerase, primers, buffer, sample DNA, pipettes and respective tips, positive controls, PCR thermocycler

Materials for DNA Detection

ELISA plates, coating buffer, streptavadine [Jackson Immuno Research], incubators, hybridization buffer, antifluoresceine, Fab fragment, antibody dilution buffer, OVS-2 FL probe, SSPE / SDS buffer, sulfuric acid

Methods

Preparation of DNA from Pools of Heads or Bodies

ISOLATION OF HEADS FROM AFRICAN BLACK FLIES

1. Begin with flies that have been preserved in isopropanol. Rinse the flies in 95% ethanol, and pour them out into a plastic container or on to a weigh paper. Allow the ethanol to evaporate until the flies appear to be dry. You can have up to 200 flies per pool. Do not allow the flies to desiccate completely, or the head purification will not work.

2. Place the flies in a clean dry 15 ml polypropylene conical centrifuge tube. You can do up to five tubes at one time.

3. Place the bottle at -70ºC, or in the vapor phase of liquid nitrogen for 30 minutes.

4. Place the tubes containing the frozen flies into the fingers of a regular latex glove. Snap off the heads by holding on to the sleeve of the glove and snapping the fingers against the bench.

5. Collect the flies from the bottle by resuspending them in 95% ethanol, and removing them with a wide bore pipet, such as a plastic transfer pipet. The amount of ethanol is not important, but try to get all of the fly material.

6. Pour the ethanol/fly mix through a 25 mesh sieve connected to a pan. The bodies will collect on the sieve, and the heads will pass through. Rinse the flies collected on the sieve to ensure that all of the heads pass through.

7. Decant the excess ethanol from the pan, using a narrow pipet. Allow the heads to dry at room temperature.

PREPARATION OF DNA FROM POOLS OF HEADS OR BODIES

1. Begin with a pool of heads or bodies. The largest pool found to successfully work with this protocol is 200 heads or bodies. Plan to prepare the DNA in batches of 18 pools. In each batch, include two sham extractions. These are empty tubes that are carried through the process exactly as if they contained flies. These are necessary to ensure that the DNA extraction process remains free of contamination.

2. Place the heads or bodies in a 1.5ml snap cap microcentrifuge tube. Rinse the flies three times in 95% ethanol. Remove as much of the ethanol as possible using a narrow tip pipet, or pipetman.

3. Allow the ethanol to evaporate for about 10 minutes at room temperature.

4. Add 200ul of 10mM Tris-HCl, 1mM EDTA (pH 8.0) (TE) to the fly heads or bodies, and place in a microfuge tube. Homogenize the heads or bodies with a disposable blue plastic homogenizer.

5. Add 40ul 10mg/ml proteinase K.

6. Incubate 2 hours at 56°C.

7. Add 20ul of 1M DTT and add 240ul TE to bring the total volume to approximately 500ul.

8. Pierce the top of the tube and incubate at 95°C for 30 minutes.

9. Freeze thaw 3 times.

10. Centrifuge the samples for 5 minutes at full speed in the microcentrifuge. Remove the supernatant.

11. Place the supernatant in a new tube. Add:
    a. 50ul 1M Tris HCl (pH 7.5)
    b. 12.5ul 4M NaCl

    to each sample.

12. Add 5ul of 0.5 uM OVS-2-biotin to each tube.

13. Heat the mix to 95°C for three minutes in the hot block and allow it to cool slowly until the temperature is <35°C.

Bead preparation and sample binding:

14. While the samples are cooling, resuspend the strepavidin magnetic beads and remove enough beads so that you have 10ul of beads per sample.

15. Pipet the beads into a flat bottom microtiter plate well into Row A of the plate, using no more than 50ul per well. Place on the magnet and allow the beads to collect for 2 minutes.

16. Wash the beads 5x with binding buffer (100mM Tris-HCl (pH 7.5) 100mM NaCl) using 200ul per wash. Allow the beads to collect for 2 minutes on the magnet between washes.

17. Resuspend the beads in the original volume of binding buffer. Add 10ul of the bead solution to each sample.

18. Incubate the samples on the tube rocker overnight at room temperature. (Note – 1-2 hours is sufficient, but we usually stop here at the end of the first day)

Washing and Elution:

19. Pipet 100ul of the samples into Rows B-H of the same flat bottom microtiter plate you used above to prepare the beads set on the magnet. Allow the beads to collect in the magnetic field for two minutes. Remove the supernatant. Sequentially pipet another 100ul of the sample into the well, allowing two minutes for the beads after each addition to collect on the side of the tube facing the magnet before removing the solution and adding the next aliquot. Do not disturb the beads during this process. In the end, all of the magnetic beads in each sample should end up held by the magnet at the bottom of each well, and all the solution should have passed through the well.

20. Wash the beads 6 times with 150ul of binding buffer per wash, using a multi-channel pipet.

21. After the last wash, remove as much solution as possible. Resuspend the beads in 20ul of PCR water. Transfer the samples (solution and beads) to a PCR plate. Seal the wells containing the samples with a plate sealer, place in the thermocycler.

22. Run the following program:
    80°C for 2 minutes

4ºC hold.

23. Place the plate on the magnet and collect the beads for two minutes. Remove the supernatant to a new plate. The solution contains your purified DNA. Store the plate at -20ºC.

Reagents and supplies:

0.5 uM OVS2 capture probe:

5´B-AATCTCAAAAAACGGGTACATA-3´, where B = biotin

Dissolve the primer in the amount of PCR water indicated on the spec sheet. This will produce a 100uM solution. To prepare the working solution mix 2ul of the 100uM stock with 398ul of PCR water. This will produce the 0.5uM working solution, enough for 80 samples.

Dynabeads® M-280 Streptavidin coated magnetic beads:Invitrogen #112-05D

DynaMag™-96 Bottom collection magnet (96 well format) Invitrogen #123-32D

TE 80 (10mM Tris-HCl (pH 8.0) 1mM EDTA)

For 100ml:

1M Tris HCl, pH 8.0	1ml
500mM EDTA pH 8.0	0.2ml

Store at room temperature

1M DTT:

1.54 g dithiothreitol (DTT)
Dissolve in 8 ml distilled water. Adjust volume to 10ml. Store at -20ºC.

Modified PCR amplification protocol for the O-150 family

Master Mix - for 100 reactions:

PCR water	3.2ml
10X buffer F	500µl
2mM each dNTP	500µl
20µM 1632 primer	125µl
20µM 1633 biotin primer	125µl
Taq polymerase	50µl
Total volume	4.75ml

For each reaction, use 45µl of the master mix plus 5µl sample DNA.Add 5µl of PCR water to the negative control wells in place of the DNA(wells A1-A10). Aliquot the master mix into the wells of a 96 well PCRplate in the PCR master mix area. Remove the plate to the DNA prep areaand add the DNA samples. Move the plate to the post PCR area and addthe positive control DNA to the positive control wells (A11 and A12). Sealthe plate with a plate sealing strip.

PCR program:

5 cycles:

1 minute @ 94°C
2 minutes @ 37°C
30 seconds @ 72°C

Then go to 35 cycles:

30 seconds @ 94°C
30 seconds @ 37°C
30 seconds @ 72°C

Finish with 6 minutes @ 72°C

10X buffer F:

Final Concentration	Stock Concentration	Amount to Add
600mM Tris-HCl (pH9.0),	1M	30ml
150mM $(NH_4)_2SO_4$	1M	7.5ml
20mM $MgCl_2$	1M	1.0ml
PCR water	---	11.5ml

Primers:

Dissolve the primer in the amount of PCR water indicated on the spec sheet.This produces a 100uM stock solution.Aliquot the stock solution into 100µl aliquots in screw capped microfuge tubes and store at -80°C.

Add 400µl of PCR water for a 20µM working solution. Store at -20ºC.

Primer sequences:

1632:5’ GATTYTTCCGRCGAANARCGC 3’
1633 biotin: 5’ biotin-GCNRTRTAAATNTGNAAATTC 3’

N = A,G,C OR TY = C OR TR = A OR G

ELISA based detection of O-150 PCR products

Preparation of PCR reactions:

Before beginning the protocol, set up a number of 10 fold serial dilutions of the positive control DNA in order to determine the limit of detection of the assay for the positive control stock you are using. Run standard 50µl PCR reactions containing 1µl of the serial dilution of the positive control DNA plus 1.5µl of PCR water. Once you determine the highest dilution that reliably gives a PCR positive result, use 2.5µl of this dilution as your positive control in all subsequent experiments.

For each complete ELISA plate, plan to set up two positive control reactions (in wells A11 and A12) and 10 negative control reactions (in rows A1-A10). The negative control reactions should contain 2.5 µl of PCR water in place of the DNA. In one of the positive control PCRs, add 2.5 µl of the positive control DNA. In the second reaction, add 2.5 µl of the PCR positive control DNA plus 2.5 µl of DNA from a preparation from a pool that has previously tested negative, or DNA prepared from a pool of 50 flies known to be uninfected. The first positive control will tell you that your reaction is working as efficiently as possible, while the second positive control (the one with the fly DNA) will demonstrate that your fly DNA preparations are not inhibiting the PCR. You should be able to do 84 PCRs on each ELISA plate.

ELISA protocol:

1. Coat plates (Immulon 2 round bottom) with 100µl of 1µg/ml Strepavidin (dilute the stock of 1 mg/ml 1/1000 in 1x Coating buffer made fresh each week) at 4^{o}C overnight.

2. In the morning, remove the hybridization buffer from the refrigerator and place in a 37^{o}C water bath to dissolve. Ensure that there is sufficient TBS/Tween wash made (2 liters per plate for the whole protocol), and a squirt bottle filled with TBS/Tween at room temperature for most washes. Ensure that the SSPE/SDS buffer is at 56^{o}C and that you have a squirt bottle of TBS/Tween also heated to 56^{o}C. Warm the BluePhos solutions to room temperature.

3. Wash plates 6 times with room temperature (RT) TBS/Tween, emptying the plate on to a paper towel between washes. Use a squirt bottle loaded with TBS/Tween for the washes.

4. Add 20µl hybridization buffer (HB) to all wells.

5. Add 5µl of undiluted PCR product (10% of the PCR reaction) to the appropriate wells.

6. Incubate 30 minutes at room temperature. During this time dilute the probe DNA (OVS2-FL) to 100 ng/ml in HB.

7. Wash the plates 6 times with RT TBS/Tween.

8. Add 100µl 1N NaOH to all wells.

9. Incubate for 1 minute at room temperature.

10. Wash plate 6 times with RT TBS/Tween.

11. Add 50 µl OVS2-FL-probe diluted to 100ng/ml in HB to all wells.

12. Incubate for 15 minutes at 42ºC.

13. Wash the plate 6 times with TBS/Tween.

14. Add 100µl 56ºC SSPE/SDS buffer to all wells.

15. Incubate 10 minutes at 56ºC. Mix equal volumes of BluePhos solutions A and Solution B (5ml of each for one plate) in 15ml conical tubes. Use within 30 minutes of mixing. Prepare the conjugate (anti flouresecin Fab fragment) by diluting it 1/10,000 in antibody dilution buffer (i.e. 1µl Fab fragment plus 10ml dilution buffer).

16. Wash the plate 6 times with TBS/Tween heated to 56ºC.

17. Add 50µl of the diluted anti-florescin Fab fragment to each well.

18. Incubate 15 minutes at 37°C.

19. Wash plate 6 times with TBS/Tween.

20. Add 100ul of freshly mixed BluePhos solution to each well. Tap gently to Mix. Incubate at room temp for 20-30 min. Blue color will start to appear.

21. Dilute one part of AP stop solution with 9 parts of high quality water to make 1X stop solution at room temperature. i.e. For one plate 1ml AP stop +9ml H_2O.

22. Add 100ul of AP stop solution to each well to stop color development at desired time.

23. Read plates at 650nm.

Data analysis:

Take the mean of all ten negative control wells and determine the standard deviation of the negative controls. Add a value equal to three standard deviations to the mean of the negative control wells.

We use two different cutoffs for the assay depending upon what the value of the mean plus 3 SD of the negatives are. If this value is above 0.1, use the mean plus 3 SD as the cutoff. If it is below 0.1, set the cutoff at 0.1. The reason for this is that as you do the assay, your consistency will improve and the blank wells will begin to show very little variation. The major source of variation that you see when this happens arises from background contributed by the DNA samples themselves and not variation in the assay. If you get very tight low blanks, the cutoff will not be sufficient to account for this underlying source of variation, and you will score a number of wells as false positives. However, this underlying variation never exceeds 0.1, so 0.1 is the cutoff to use in this situation.

Any well with an OD value below the cutoff is scored as negative and anything with a value above the cutoff is scored as a putative positive. When a putative positive is detected, repeat the entire assay, beginning with a new PCR reaction. Any sample that scores above the cutoff in two independent PCRs is scored as a confirmed positive.

Materials and solutions:

Microtiter plates: Immulon 2 round bottom 96 well plates. Dynex order# 3655.

Strepavidin: Jackson Immunoresearch

Coating Buffer: 50mM $NaHCO_3$, 2mM $Na2CO_3$. (4.2g Sodium bicarbonate, plus 0.211g Sodium carbonate per liter).Make the coating buffer fresh weekly as the pH goes off during storage. Store it at 4^oC

TBS/Tween: 1X TBS, 0.05% Tween 20.

Stocks needed:

10x TBS:

30.2g Tris base
43.8g NaCl

Dissolve in 350ml distilled water. Adjust pH to 7.5 with HCl. Adjust volume to 500ml.

Store at room temperature

TBS/Tween (for 2 liters):

10X TBS	200ml
Twen 20	1ml

Store at room temperature

Hybridization buffer (HB):

Stocks needed:

20X SSPE:

174g NaCl

27.6g NaH_2PO_4

7.4g disodium EDTA

dissolve in 700ml distilled water. Adjust pH to 7.4 with NaOH. Bring volume to 1 liter.

Store at room temperature

10X Denhardt's:

0.2% (w/v) bovine serum albumin

0.2% (w/v) polyvinylpyrrolidone

0.2% (w/v) Ficoll 400.

Store at -20^{o}C

1% N-lauryl sarcosine:

For 100ml:

N-lauryl sarcosine 1g

dissolve in 80ml distilled water. Adjust volume to 100ml

Store at room temperature

For 500ml HB:

120ml 20X SSPE

50ml 10X Denhardt's

50ml 1% (w/v) N-lauryl sarcosine

1ml 10% SDS

Water to 500ml

Store at 4^{o}C

1N NaOH:

Weigh out 0.4g solid NaOH. Dissolve in 8 ml distilled water. Adjust volume to 10ml.

Make fresh daily.

OVS2-FL probe

We order this from Operon (Fisher), although products from other suppliers will probably work as well.

5' AATCTCAAAAAACGGGTACATA-fl 3' where fl = flourescein.

Prepare a master solution at 100 µg/ml concentration. This is a 1000x stock, so dilute 1ul/ml of water before using to make the working solution. Store in the dark at -20^{o}C.

Antibody dilution buffer:

0.4M NaCl, 0.1M Tris-HCl (pH 7.5) 0.5% (w/v) bovine serum albumin. Store at 4^{o}C.

For 100ml:

4M NaCl	10ml
1M Tris HCl (pH 7.5)	10ml
BSA	0.5g
Distilled water	80ml

Dissolve BSA. Store at 4^{o}C

SSPE/SDS Buffer:

1XSSPE, 0.1% SDS

For 200ml

20x SSPE	10ml
10% SDS	2ml
distilled water	188ml

Store at 42°C sealed tightly. Shake bottle before use.

Anti-flourescein-AP Fab fragments:

Order number 1426338 from Roche Biochemicals

Blue Phos detection system:

catalog # 50-88-22 from KPL www.kpl.com

APStop solution catalog # 50-89-00 from KPL www.kpl.com

Quality Control

1. The positive control must turn positive and negative controls must turn negative for the assay to be valid.
2. Repeat every positive sample to confirm to positivity.

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APPENDIX 9: A TAXONOMIC RESOURCE FOR THE STUDY OF ANTHROPOPHILIC BLACKFLIES IN ETHIOPIA

CONTENTS

TABLE 1:

Species of Blackflies (Diptera: Simuliidae) Recorded from Ethiopia

Simulium (*Anasolen*) *dentulosum*
Simulium (*Anasolen*) *nili*
Simulium (*Anasolen*) *shoae*

Simulium (*Edwardsellum*) *damnosum* Kisiwani (?)
Simulium (*Edwardsellum*) *damnosum* Kisiwani E
Simulium (*Edwardsellum*) *kaffaense*
Simulium (*Edwardsellum*) *kulfoense*
Simulium (*Edwardsellum*) *soderense*
Simulium (*Edwardsellum*) *damnosum* 'Gondar Form'

Simulium (*Lewisellum*) *ethiopense*
Simulium (*Lewisellum*) *nyasalandicum* (?)

Simulium (*Meilloniellum*) *adersi*
Simulium (*Meilloniellum*) *hirsutum*
Simulium (*Meilloniellum*) *sexiens*
Simulium (*Meilloniellum*) *urundiense* (?)

Simulium (*Metomphalus*) *bovis*
Simulium (*Metomphalus*) *dawaense*
Simulium (*Metomphalus*) *gibense*
Simulium (*Metomphalus*) *jimmaense*
Simulium (*Metomphalus*) *hargreavesi*
Simulium (*Metomphalus*) *medusaeforme*
Simulium (*Metomphalus*) *vorax*

Simulium (*Nevermannia*) *arabicum*
Simulium (*Nevermannia*) *brachium*
Simulium (*Nevermannia*) *buckleyi*

Simulium (*Nevermannia*) *ruficorne*

Simulium (*Pomeroyellum*) *alcocki*
Simulium (*Pomeroyellum*) *geigyi*
Simulium (*Pomeroyellum*) *impukane*
Simulium (*Pomeroyellum*) *johannae*
Simulium (*Pomeroyellum*) *tentaculum*
Simulium (*Pomeroyellum*) *weyeri*
Simulium (*Pomeroyellum*) *cervicornutum*
Simulium (*Pomeroyellum*) *unicornutum*
Simulium (*Pomeroyellum*) *awashense*
Simulium (*Pomeroyellum*) *mcmahoni*
Simulium (*Pomeroyellum*) 'Ethiopian A'
Simulium (*Pomeroyellum*) 'Ethiopian B'
Simulium (*Pomeroyellum*) 'Ethiopian C'
Data from Adler & Crosskey (2014)

TABLE 2:

Anthropophilic Species and the Vectors of Onchocerciasis in Ethiopia

Anthropophilic Species	Status in Ethiopia
Simulium (*Anasolen*) *dentulosum*	✓✓
Simulium (*Anasolen*) *shoae*	✓✓
Simulium (*Edwardsellum*) *kaffaense*	✓✓✓
Simulium (*Edwardsellum*) *damnosum* subcomplex 'Gondar Form'	(✓✓✓)
Simulium (*Lewisellum*) *ethiopense*	(✓✓✓)
Simulium (*Meilloniellum*) *adersi*	✓✓
Simulium (*Metomphalus*) *bovis*	✓
Simulium (*Metomphalus*) *gibense*	✓✓
Simulium (*Metomphalus*) *vorax*	✓

Notes:

This list contains species which have been recorded from Ethiopia and have been recorded as anthropophilic in at least part of their range, but not necessarily in Ethiopia.

✓ - Present in Ethiopia (but not recorded as anthropophilic in Ethiopia)

✓✓ - Recorded as anthropophilic in Ethiopia

✓✓✓ - Vector of onchocerciasis in Ethiopia

(✓✓✓) - Probably a vector of onchocerciasis in Ethiopia

Key 1:

Identification of all Life Stages of the *S. damnosum* Complex

(For the identification of adult females, pupae and larvae of *S. damnosum* s.l. from all other African Simuliidae)

1.1.ADULT FEMALES

***Simulium damnosum* s.l.:** -Fore tarsus broad and flattened with a conspicuous dorsal crest of hairs (see arrow in Figure 1).Legs all black except for a conspicuous creamy-white band across middle of hind basitarsus (see arrow in Figure 1).

Other African species: -Fore tarsus not conspicuously dilated and without obvious hair crest.Legs can be any pattern, usually unicolorous black, extensively pale or mixed dark and pale.

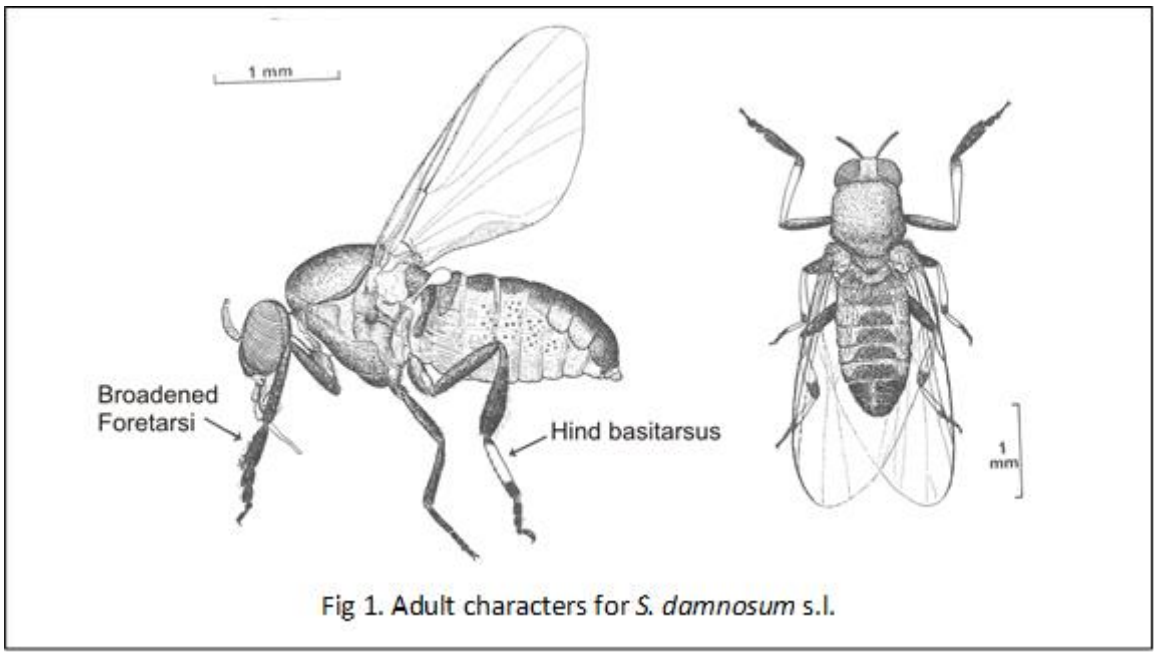


Fig 1. Adult characters for *S. damnosum* s.l.

1.2.PUPAE

***Simulium damnosum* s.l.:** -Pupal Gill as in Figure 2, composed of inflated thin-walled tubular branches and having some resemblance to a bunch of bananas.Branches arranged as a pair of large basal arms and three curved outer branches and six weaker inner branches, the latter arising as three pairs from short common stalks.

Other African species: -Pupal gills different from this description.Other forms of the gills in other species are immensely varied, but *S. neavei* is shown as an example (See Fig 2).

1.3.LARVAE

***Simulium damnosum* s.l.:** -Larval thorax with dense covering of many small black setae and scales which always extend onto the proleg (Fig 2). With characteristic paired dorso-lateral tubercles (Fig 2), which can be large and easily seen or small and only visible under the microscope.

Other African species: - Never with setae/scales on the proleg and without dorso-lateral tubercles.

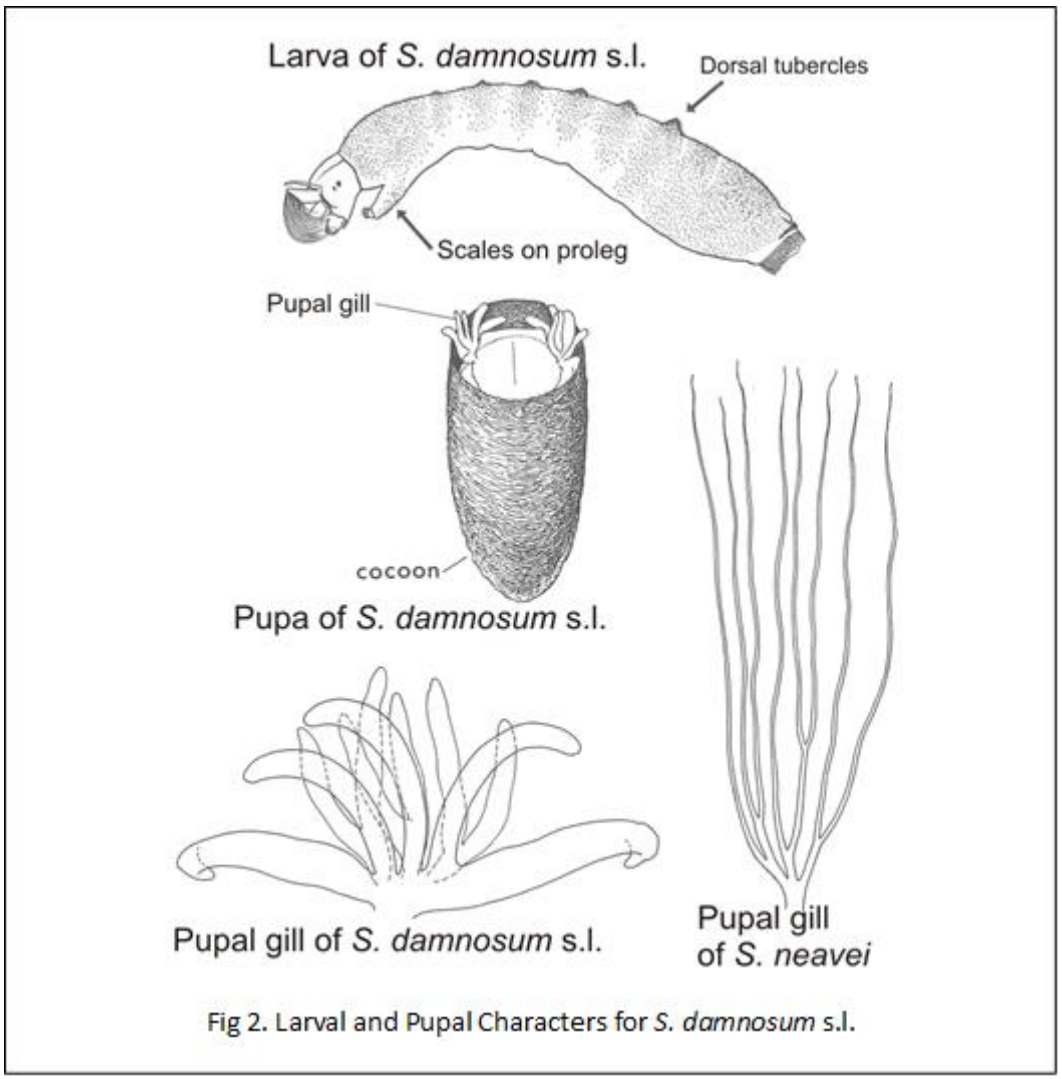


Fig 2. Larval and Pupal Characters for *S. damnosum* s.l.

Key 2:

Identification of Anthropophilic Blackflies and Vectors of Onchocerciasis in Ethiopia

For the identification of adult female vectors (*S. damnosum* s.l. and *S. ethiopiense*) as well as all other blackflies which might be caught landing on humans in Ethiopia.

Start with question 1:

Question 1. Your specimen must be like either 1a or 1b.

1a. Fore tarsi broad and flattened with conspicuous dorsal crest of hairs.Legs entirely dark except for large creamy-white band on hind basitarsus.Sides of abdomen with silver or silver-yellow scales arranged in clumps.(Figures 1 and 2)

.....Specimen is: ***Simulium damnosum* complex**

1b. Fore tarsi not conspicuously broad (Figure 2).Legs all dark or with various colour patterns (but not as above).Any scales on sides of abdomen not grouped into clumps.

.....**Go to question 2**

Question 2. Your specimen must be like either 2a or 2b.

2a.Pleural membrane (Figure 1) without hairs.

.....**Go to question 3**

2b.Pleural membrane with hairs (the hairs may be only a small patch near the top of the membrane and should be examined under a dissecting microscope with good light.They may be missing in old specimens).

..... Specimen is:**a non-vector species**

[= *S. adersi*, *S. dentulosum*, *S. shoae*[1] or *S. gibense*]

Go to question 4

Question 3. Your specimen must be like either 3a or 3b.

3a. Large black species with silvery-yellow or golden scales on thorax and abdomen (sometimes also with black scales).Thorax without three-lined dorsal pattern (but it might show a pattern of scales).Wing length 2.6-3.6 mm.

.....***Simulium ethiopiense***

3b. Smaller[2] grey or brown-black species, also with silvery, yellow or golden scales on thorax and abdomen.Thorax with pattern of three dark longitudinal lines on dorsum (Figure 3). Wing length usually 1.3-2.65 mm.

..... Specimen is:**a non-vector species**

[= *S. bovis* or *S. vorax*]

Go to question 5

Question 4. Your specimen must be like either 4a, 4b or 4c.

4a. Claws with large (easily seen) basal tooth (Figure 4) (a small silvery-grey species)

.....***Simulium adersi***

4b. Claws with very small (inconspicuous) basal tooth (Figure 5).Thorax dull black, without pattern of three dark longitudinal lines on dorsum (although there may be a pattern of silvery, yellow or golden scales). (larger grey-black species usually in swift flowing mountain streams)

.....***Simulium dentulosum***

+ *Simulium shoae*[1]

4c. Claws without basal tooth (similar to Figure 6) and thorax with pattern of three dark longitudinal lines on dorsum (similar to Figure 3).

..... ***Simulium gibense***

[1] Note: *Simulium shoae* was originally described as a larval and pupal form of *S. dentulosum*. The adult female has not been described, but is here assumed to be similar to *S. dentulosum*.

[2] Note: *S. vorax* is a larger species (wing length 2.5-3.5 mm), but it is still grey with the dorsal lines on the thorax.

Question 5. Your specimen must be like either 5a or 5b.

5a. Scutum with brownish patches on either side of the three dark longitudinal lines (Figure 3)

.....***Simulium vorax***

5b. Scutum (dorsum of thorax) without brownish patches on either side of the three dark longitudinal lines (Figure 3)

.....***Simulium bovis***

FIGURES FOR KEY 2:

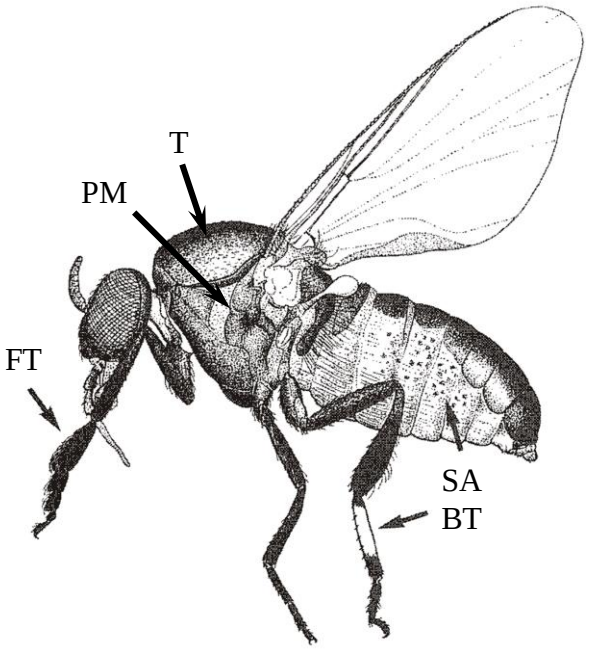
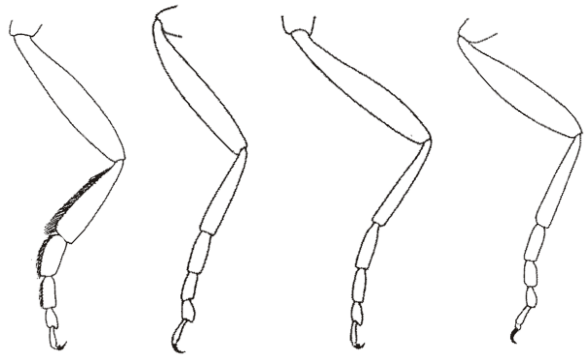
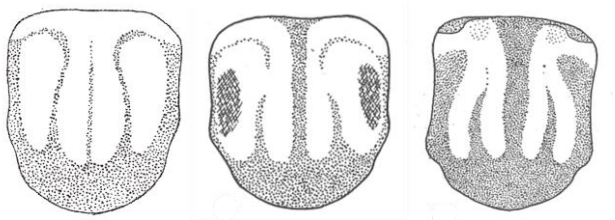


Figure 1: Lateral view of adult female *S. damnosum* s.l. showing swollen fore tarsi (FT), banded hind basitarsus (BT), clumps of scales on sides of abdomen (SA), and position of the thorax (T) and the pleural membrane (PM).



S. damnosum *S.neavei* *S.Vorax* *S.griseicolle*

Figure 2: Front leg of *S. damnosum* showing swollen fore tarsi in relation to three other example species



S. medusaeforme *S. vorax* *S. bovis*

Figure 3: Examples of three-lined patterns on thorax (dorsal view with anterior end at top)

Figure 4: Claw (at end of leg) from *S. adersi* showing large basal tooth.

Figure 5: Claw from *Simulium dentulosum* with inconspicuous basal tooth.

Figure 6: Claw (enlarged) from subgenus *metomphallus* with no basal tooth, and showing position of claw at end of leg.

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APPENDIX 10. ROLES AND RESPONSIBILITIES OF PARTNERS IN ONCHOCERCIASIS ELIMINATION

Partner	Roles and responsibilities
Federal Ministry of Health (FMoH)	Develops strategies and guidelines for implementation
	Coordinates partners involved in Onchocerciasis elimination
	Ensures timely submission of treatment report and Mectizan application
	Ensures Mectizan is imported at least one month before MDA schedule
	Ensures timely distribution of Mectizan tablets to endemic Regions/Zones
	Ensures proper documentation of Mectizan treatment coverage
	Coordinates efforts to map the distribution of Onchocerciasis in the country
	Establishes cross border collaboration with concerned neighboring countries
	Establishes Onchocerciasis ExpertAdvisory Committee and National Verification Committee and ensures they function properly
	Co-ordinates and invites (as found necessary) experts in the field to conduct periodic assessment and determine status of the elimination program

Partner	Roles and responsibilities
Food, Medicine and Health Care Administration and Control Authority (FMHACA)	Works closely with FMoH and PFSA to expedite the importation of Mectizan
Pharmaceuticals Funding and Supplies Agency (PFSA)	Works closely with FMoH and FMHACA to expedite the importation of Mectizan
Ethiopian Public Health Institute EPHI)	Conduct Polymerase Chain Reaction (PCR) using the O150 primer to determine *Onchocerca volvulus* DNA present in the vectors blackflies (*Simulium* species), and calculate infection and infection rates using the PoolScreen algorithm. Conduct *Onchocerca volvulus* antibody test using the OV16 recombinant antigen in ELISA based assays (or other similar assays) using finger prick blood samples (collected from onchocerciasis endemic areas) in order to determine exposure to *O. volvulus*. Conduct when necessary the confirmation of active infection in persons with OV16 positive blood samples using O150 PCR testing of skin snips in order to verify the presence of *Onchocerca volvulus* DNA. Apply any other test listed as appropriate under the World Health Organization's (WHO) verification guidelines. Ensures proper documentation of REMO data and epidemiological survey results

Partner	Roles and responsibilities
Ethiopia Mapping Agency (EMA)	Provide updated shape/digital maps of Ethiopia.
Regional Health Bureaus	Provide support to Zone Health Departments to ensure that reports are submitted timely, MDA is conducted as per the schedule and drug balance is maintained properly Support and facilitate operational researches, surveys, assessments related to Onchocerciasis elimination Co-ordinate partners working on the onchocerciasis elimination in the specific region
Zonal Health Departments	Provide support to Woreda Health Offices to ensure that reports are submitted timely, training is conducted as per the manual , MDA is conducted as per the schedule and drug balance is maintained properly Keep a record of zonal Mectizan treatment data
Woreda Health Offices	Lead the training and deployment of HEWs who go to their respective kebeles and orient Health Development Army about onchocerciasis elimination Ensure that MDA is done as per the schedule (annual or biannual) Maintain record of Woreda Mectizan treatment data
Health Extension Workers	Train and orient Women HDA HDA/CDDs to improve monitoring to achieve maximum therapeutic coverage; improve documentation and reporting

Partner	Roles and responsibilities
	Keep a record of Mectizan treatment data at Kebele level
World Health Organization/APOC	The preparation of Ethiopia for verification of onchocerciasis elimination is a multi-faceted process involving many stakeholders.It is envisaged that the development partners, andespecially the WHO, will continue to provide technical support in the form of consultants from time to time to not only strengthen the national capacity but also steer the process of preparedness for verification.
	Work closely with FMoH and other partners to ensure drug delivery as per the schedule;
	Ensure that resource allocated for projects is disbursed at the appropriate time.

Partner	Roles and responsibilities
The Carter Center	As a technical partner of EPHI shall provide technical advice through its technical personnel, reference laboratory, and expert consultants. The Carter Center shall supply the necessary reagents, materials and consumables for O150 PCR and OV16 ELISA testing Research data/materials shared with reference laboratory outside the Ethiopia should be as per the memorandum of understandingandMaterials Transfer Agreement signed between institutions/organizations Provide the needed consultation (either in Ethiopia or via telecommunications) to ensure that all laboratory personnel are well trained, high quality control standards are maintained, and validated results are generated by the Onchocerciasis Molecular Laboratory. Provide consultants in the field as recommended by the EOEEAC or based on program needs as discussed within the TCC-assisted program or the National Onchocerciasis Elimination Taskforce.
Light For the World	Strengthen its support to ZHDs and WoHO to ensure timely MDA with high treatment coverage

Partner	Roles and responsibilities
Academic and Research Institutions	Strengthen its support to ZHDs and WoHO to ensure timely MDA with high treatment coverage Work closely with FMoH and its partners to produce the evidence needed to start as well as stop MDA with Mectizan Actively involved in monitoring and evaluation of Onchocerciasis elimination program and operational research related to onchocerciasis elimination

APPENDIX 11. CURRENT STATUS OF ONCHOCERCIASIS OF ELIMINATION PROGRAM OF ETHIOPIA (2014)

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
Semi-annual TX	Kaffa	11	2001	1,075,272	Semi-annual	2013	TCC
	Sheka	5	2001	243,907	Semi-annual	2013	TCC
	Bench Maji	11	2003	805,050	Semi-annual	2013	TCC
	Jimma	14	2013	2,142,471	Semi-annual	2013	TCC
	Illubabor	12	2013	721,731	Semi-annual	2013	TCC
	North Gondar	4	2013	654,742	Semi-annual	2013	TCC
				5,643,173			
If assessments Indicate ongoing transmission semi-annual TX will be launched in 2015	Jimma	4	2004	891,037	Annual		TCC
	Illubabor	13	2004	907,092	Annual		TCC
	North Gondar	Gendewuha	2003	374,810	Annual		TCC
	Metekel	Guba	2004	159,681	Annual		TCC
	Gambella	5	2004	176,693	Annual		TCC
	Kellem Wollega	10	2005	932,208	Annual		LFW

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
	West Wollega	Gimbi	2005	132,053	Annual		LFW
		Homa	2005	40,719	Annual		LFW
	East Wollega	9	2005	782,404	Annual		LFW
	West Shewa	Bako Tibe	2005	121,706	Annual		LFW
Subtotal				**4,518,403**			
	Gambella	2	New	68,562	Semi-annual	2013	TCC
	West Wollega	17	New	1,350,415	Semi-annual		*LFW*
	East Wollega	8	New	110,688	Semi-annual		*LFW*
	Horro Gudru	6	New	441,289	Semi-annual		*LFW*
	West Shewa	5	New	496,623	Semi-annual		*LFW*
	Metekel	3	New	176,178	Semi-annual		TCC
	Kamashi	6	2014	159,825	Semi-annual	2014	RTI/ENVISION
	Assossa	7	2014	326,575	Semi-annual	2014	RTI/ENVISION
Subtotal				**3,130,155**			
	Dawro	Tercha	New	126,330	Semi-annual	2015	TCC

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
Planned semi-annual TX for 2015	Konta	Konta	New	112,068	Semi-annual	2015	TCC
	East Gojjam		New	2,302,828	Semi-annual	2015	TCC
	West Gojjam		New	2,254,745	Semi-annual	2015	TCC
	Awi	Jawi	New	8,868	Semi-annual	2015	TCC
Subtotal				**4,804,839**			
No Intervention and Not Yet Mapped	North Shewa			1,621,167			
	East Shewa			1,521,978			
	Arsi			2,954,793			
	West Hararge			2,107,068			
	East Hararge			3,071,251			
	Bale			1,590,751			
	Borena			1,083,549			
	Guji			1,584,145			
	West Arsi			2,214,591			
	Shinile			505,787			
	Jijiga			1,070,686			

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
	Degehabur			529,426			
	Warder			338,627			
	Korahe			345,956			
	Fik			385,372			
	Gode			513,924			
	Afder			632,026			
	Liben			597,334			
	North Western Tigray			812,352			
	Central Tigray			1,374,493			
	Eastern Tigray			834,077			
	Southern Tigray			1,108,844			
	Western Tigray			394,663			
	Mekele special			237,922			
	Afar Zone 1			460,150			
	Afar Zone 2			383,393			

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
	Afar Zone 3			216,693			
	Afar Zone 4			278,783			
	Afar Zone 5			200,408			
	South Gondar			2,190,006			
	North Wollo			1,608,143			
	South Wollo			2,695,191			
	North Shewa			1,967,372			
	Waghimra			455,756			
	Awi			1,049,954			
	Oromia			491,923			
	Bahir Dar Special			235,714			
	Argoba spl woreda			37,916			
	Gurage			1,435,606			
	Hadiya			1,394,452			
	Kembata Tembaro			765,929			
	Sidama			3,326,045			

Current Status of Interventions	Zone	# of Districts	Year MDA started	Total population	Type of MDA treatment	Year when Semi-annual TX was launched	Assisting NGDO
	Gedeo			986,326			
	Wolayita			1,713,005			
	South Omo			647,655			
	Gamo Gofa			1,788,864			
	Dawro			552,435			
	Silti			842,158			
				53,154,659			

APPENDIX 12: ETHIOPIA ONCHOCERCIASIS ELIMINATION EXPERT ADVISORY COMMITTEE (EOEEAC)TERMS OF REFERENCE AND PROCEDURES OF OPERATION

Ethiopia Onchocerciasis Elimination Expert Advisory Committee (EOEEAC)
Terms of Reference and Procedures of Operation

I. The Ethiopian Program for Elimination of Onchocerciasis (EPEO)

This is a multi-approach program using targeted annual or semi-annual treatment with ivermectin (Mectizan®) and vector control/vector as appropriate dedicated to the goal of eliminating onchocerciasis in all the foci in the country. The Government of Ethiopia has taken the decision to change the onchocerciasis (River Blindness) control policy to phased countrywide onchocerciasis elimination in 2012with the highest political and financial support in the country.

EPEO is a program of the Ministry of Health (MOH), affected districts and communities of Ethiopia. The current key implementing partners supporting this program are WHO/APOC, The Carter Center, Lions Club, Light for the World, and RTI Envision/USAID.

Elimination of onchocerciasis in Ethiopia will entail:

- Interruption of transmission by 2020, and eventually WHO verification of elimination of onchocerciasis.
- Rigorous satisfaction of elimination certification criteria (indicators for elimination) which will be established and periodically reviewed in detail, focus by focus, by the Ethiopian Onchocerciasis Elimination Expert Advisory Committee (EOEEAC), a group of national and international experts convened under the auspices of the EPEO of Ministry of Health, Ethiopia. The criteria used will be developed by the EOEEACdrawing on the existing criteria and protocols developed by WHO, APOC, OEPA, and Uganda.

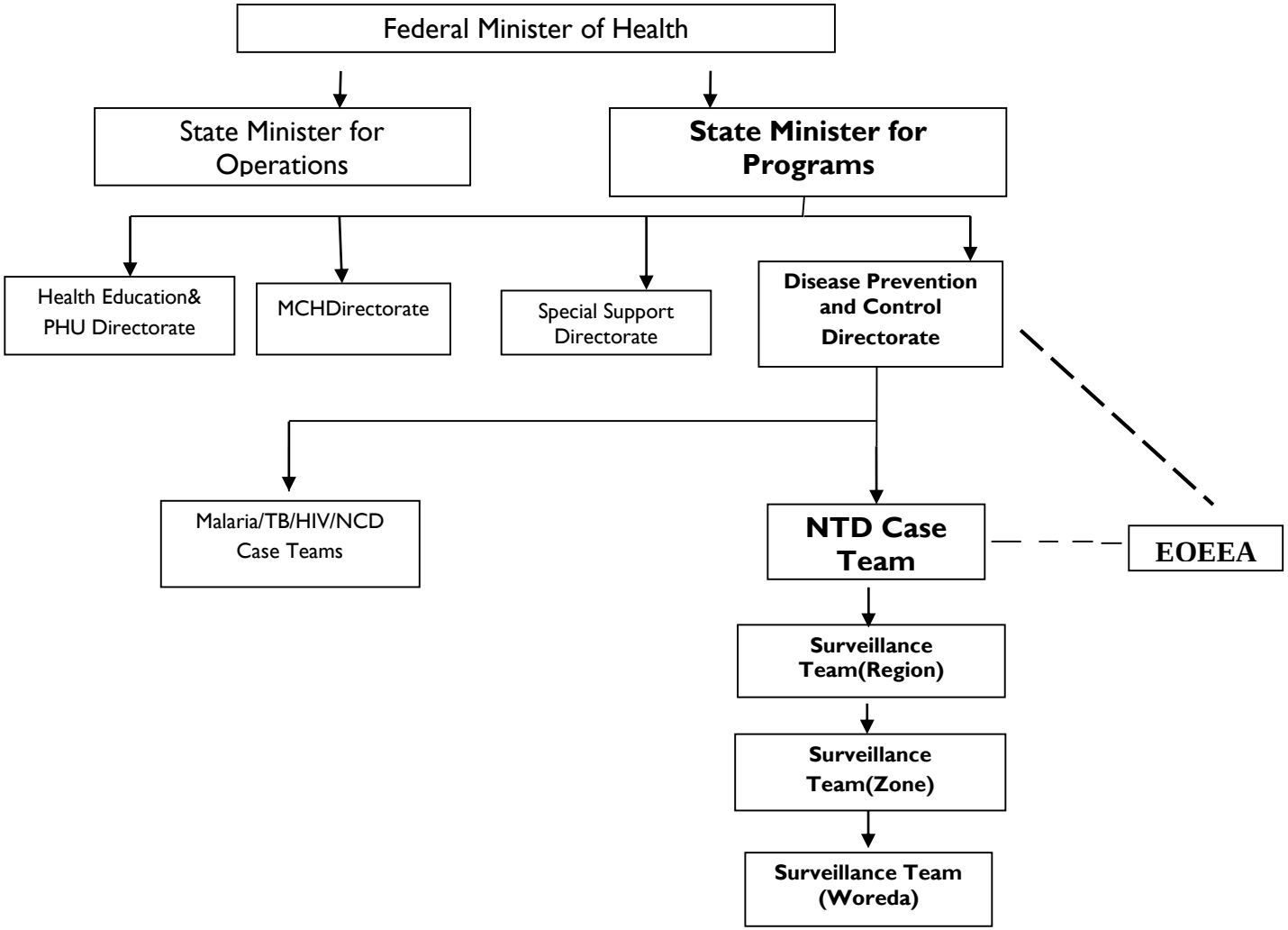
II. Ethiopia Onchocerciasis Elimination Expert Advisory Committee (EOEEAC)

Ethiopia Onchocerciasis Elimination Expert Advisory Committee (EOEEAC) is an EPEO technical advisory committeecomposed of national and international experts that conducts annual review of activities, and recommends to the EPEO effective approaches and methods for hastening onchocerciasis elimination. The EOEEAC is formed in order to provide credible, independent and international technical support for the government policy on onchocerciasis elimination.

The secretariat for theEOEEACwill bebased at the MOH's NTDs Case Team office under the direction of Federal Coordinator for EPEO.

The NTDs/Oncho Program Coordinator at the Federal Ministry of Health serves as the Secretary of EOEEAC. The implementing partner who is willing and ready to support the EOEEAC will provide a Co-Secretary to the EOEEACwho works for a three-year term. In case there is more than one supporting organization, the Co-Secretary position will revolve based on the consensus reached by the members of the EOEEAC.This arrangement is critical for ensuring continuity in the face of frequent transfers of key program personnel, and sharing of workload so that the committee receives and disseminates accurate and timely critical information for program implementation.

Organogram for the Ethiopian Program for Elimination of Onchocerciasis (EPEO)



III. Annual EOEEAC Meetings

The EOEEAC will meet for at least three days, once a year. However, an extraordinary meeting can be called to attend to urgent matters of concern.

The site of all meeting will be in Ethiopia and the place will be decided by the Secretariat in consultation with the Chairperson.

IV. EOEEAC Responsibilities

1. EOEEAC agenda will include reports on programmatic activities in each targeted focus, data analysis, certification activities, and financial status of the EPEO.
2. Scientific presentations of research and new tools relevant to EPEO, and updates on regional programs (APOC/PENDA, OEPA, NTDs, etc.), will be given during the meeting.
3. The EOEEAC has the responsibility to review national plans and other relevant onchocerciasis related proposals and tools (e.g. operations research) submitted for technical assistance to determine their viability, and to make recommendations on design and operational issues.
4. The EOEEAC serves as a forum for the partners to discuss key issues related to the national initiative.
5. The EOEEAC plays a general role in prioritizing activities, non-routine activities toward national goals.
6. The EOEEAC shall play a general ad hoc role in stimulating interest in sustained support for the Ethiopia onchocerciasis elimination initiative and have an advocacy role and the dissemination of appropriate information. The committee will also encourage and assist where necessary, publication of results in peer reviewed journals.

V. The Role of the EOEEAC Chairperson

The EOEEAC Chairperson will be elected by fellow members. The term of service is three years with the option of the EOEEAC to renew the Chairperson's tenure for additional one term.

The Chairperson will:

1. Consult with the EOEEAC members (including the Co-Secretaries) on a regular basis and communicate directly with EOEEAC members on related issues.
2. Develop the agenda in consultation with EOEEAC members, Co-Secretaries and

other relevant parties.

3. Chairs all EOEEAC meetings.
4. Receive reports from eachad hoc sub-committee.

VI. Roles of the EOEEAC Secretaries

There will be two Co-Secretaries to the EOEEAC, the Federal Coordinator of EPEO (a government employee of the Federal Ministry of Health (MOH) and representative from the partner organization as mentioned above under Article II.

1. The co-secretaries will be non-voting members of the EOEEAC.
2. They will work with the Chairperson of the EOEEAC to develop agendas, organize all aspects of the meetings and provide minutes.
3. The Secretaries shall participate fully in all EOEEAC meetings.
4. Only the Secretary from FMoH has the right to cast votes in case of a deadlock.

VII. EOEEAC Composition

1. The EOEEAC shall consist of eighteen voting members and two non-voting co-secretaries.The official members includethree from Federal Ministry of Health(including one from EPHI), one from the Ethiopian Mapping Agency, one representative from each affected regions, one from each oncho implementing partners,four at-largeindependent members who will be selected for their professional expertise in tropical diseases/oncho, finance and/or administration, and will include national and international experts.
2. WHO, African Program for Onchocerciasis Control (APOC), Mectizan Donation Program (MDP), and Lions Clubs International Foundation (LCIF/Lions Clubs of Ethiopia) shall have an observer status.
3. The World Health Organization (WHO) representative in Ethiopia will be representing the verification agency; hence, will attend EOEEAC meetings as an observer.
4. The EOEEAC as it may find appropriate will ensure that at each meeting the representatives of endemic zones are in attendance as observers.
5. Institutional and at-large members have equal voting privileges.

<u>Following constitute the EOEEAC</u>:

1. Federal Ministry of Health (2)

1a. EPHI (1)

2. Regional Health Bureaus [(5) Amhara, Oromia, SNNPR,Benishangul Gumuz, Gambella)]
3. Ethiopian Mapping Agency (1)
4. The Carter Center (1)
5. Light for the World (1)
6. RTIEnvision (1)
7. Aklilu Lemma Institute of Pathobiology, Addis Ababa University (1)
8. University of Gondar (1)
9. At-large (4)

Non-voting members:

1. Co-Secretary from FMoH
2. Co-Secretary from Oncho implementing partner who is ready and willing to support the committee

VIII. Election of EOEEAC members, and Terms of Service

1. Institutional members are selected by their agencies and serve at the discretion of their institution.
2. At-large members are nominated for service by the EOEEAC and approved by the FMoH.
3. Simple majority vote of a quorum of EOEEAC members is sufficient to elect at-large members.
4. The chairperson of EOEEAC will be elected from the voting members.
5. Term of service for at-large EOEEAC members is three years, with a two-term limit if elected for a consecutive term.The inclusion of at-large members shall be timed to stagger completion of terms.
6. Ten EOEEAC members shall make a quorum.

IX. EOEEAC Travel and Per Diem Policy

The partner supporting EOEEAC will support travel of EOEEACnational and at-large members, who will be reimbursed for reasonable transportation costs and given an adequate per diem allowance to cover hotel, meals and incidental expenses, in accordance with The Carter Center policies. However, EOEEAC members belonging

to international organizationsand partners will arrange to have these costs covered by their respective organizations.

X. Approval of EOEEAC Recommendations by the Government (Federal Ministry of Health)

Since onchocerciasis elimination process requires quick response to technical issues that may need adjustments in field plans and financial commitment, rapid and clear responses to EOEEAC recommendations based on objectively collected and analyzed data by the Federal Ministry of Health should be the norm.

As a great tool for advocacy, and motivation of communities, districts, Ministry of Health personnel and partners, the government without delay shall announce achievements attained as far as suppression or interruption, halting of interventions, commencement of post treatment surveillance (PTS) and elimination of transmission of onchocerciasis in foci when and where it is scientifically proven.

Accepted at the First EOEEAC Meeting

6th of October 2014, Addis Ababa