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Review

Diagnostic Tools for Onchocerciasis Elimination Programs

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Onchocerciasis (river blindness) is a major public health problem in sub-Saharan Africa. Major disease-control programs have greatly reduced both disease and infection prevalence by mass distribution of donated ivermectin. Recent studies have shown that local elimination was achieved in some areas following many years of ivermectin. The global health community has recently decided to build on these successes with a new program that aims to eliminate onchocerciasis. Diagnostic tests that were useful for identifying priority areas for disease prevention may not be adequate tools for elimination programs. This paper reviews available and emerging diagnostic tests for onchocerciasis and considers how they might be best employed during different stages of onchocerciasis elimination programs.

Trends

New diagnostic approaches are needed for onchocerciasis elimination programs.

This paper reviews available and emerging diagnostic tests for onchocerciasis.

Different tests may be required for different stages of elimination programs.

Additional research is needed for mapping hypoendemic areas and on establishing endpoints.

Onchocerciasis Control and Elimination Programs in Africa

Onchocerciasis is a vector-borne disease that is caused by the filarial nematode parasite *Onchocerca volvulus*. Most of the estimated 37 million people directly affected by this parasite live in 31 countries in sub-Saharan Africa (Figure 1), but there are also small foci of infection in Latin America and Yemen (www.who.int/mediacentre/factsheets/fs374/en/). *O. volvulus* infection is transmitted by *Simulium* black flies, and it can cause severe eye disease (including blindness) and skin disease; it has also been associated with excess mortality in the human host [1,2].

Several major public health programs and technical developments have greatly improved the global onchocerciasis situation since the 1970s when the Onchocerciasis Control Program (OCP) was initiated in West Africa. The OCP initially relied exclusively on larvicidal insecticides to control the black fly vectors and to reduce transmission of the parasite. The program focused on the savanna areas in 11 countries, where ocular disease and blindness due to *O. volvulus* infection were most prevalent. Following the introduction of ivermectin (Mectizan® from Merck and Co.) in the late 1980s the OCP also supported the distribution of ivermectin. While ivermectin has good activity against the **microfilariae** (Mf) (see Glossary) that cause disease in the skin and the eye, it does not kill adult *O. volvulus* worms that have an estimated reproductive lifespan of 10 years [3]; adult female worms resume production of Mf that repopulate the skin several months after ivermectin treatment. However, community-directed treatment with ivermectin (CDTI) (typically once per year) reduces disease in endemic areas by reducing Mf prevalence and by reducing the concentration of Mf in the target organs (skin and eye) [4,5].

The West African OCP ended in 2002. It overlapped several years with the African Program for Onchocherciasis Control (APOC), which coordinated CDTI in 19 African countries between 1995 and 2006. In 2006, four countries that previously participated in OCP (Ivory Coast, Ghana,

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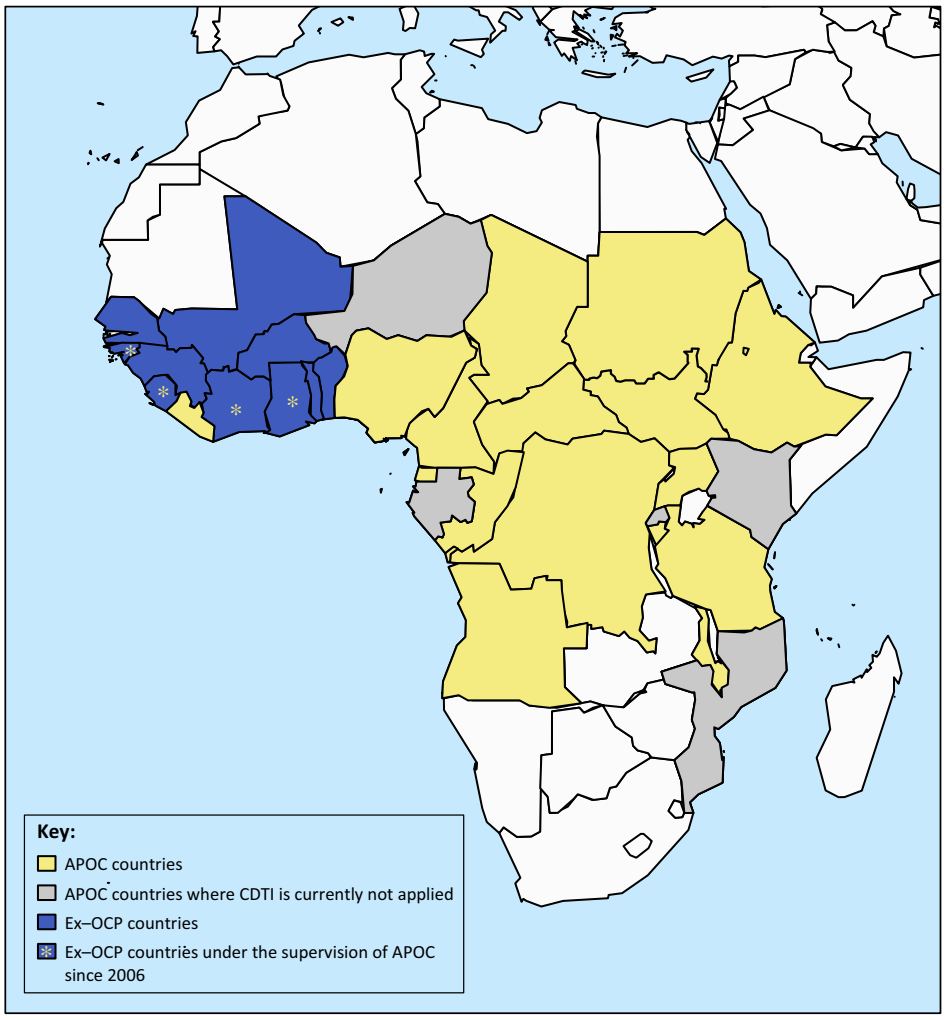

Figure 1. Map Showing the 31 African Countries Participating in African Program for Onchocerciasis Control (APOC). Original APOC countries are colored yellow. The APOC countries where community-directed treatment with ivermectin (CDTI) is currently not applied are shown in grey. The former Onchocerciasis Control Program (OCP) countries are colored in blue. Four ex-OCP countries that were added to APOC in 2006 are marked with a yellow star.

Glossary

Antigenemia: in this context this refers to the presence of parasite antigens in the blood.
Diethylcarbamazine (DEC): an anthelmintic drug.
Loop-mediated isothermal amplification (LAMP): a method for amplifying DNA.
Lymphatic filariasis (LF): infection and disease caused by *Wuchereria* or *Brugia* filarial worms.
Loiasis: an infection caused by the filarial nematode *Loa loa*.
Microfilariae (Mf): early life stage filarial larvae released by adult female worms. Mf live in the skin and are ingested by black fly vectors.
Molecular xenomonitoring (MX): uses molecular techniques to detect parasite DNA in vector species (for example, *Simulium* black flies).
Rapid epidemiological mapping of onchocerciasis (REMO): a mapping tool based on nodule prevalence in adults.
Sowda: a form or onchocerciasis characterized by severe immune-mediated skin disease in a localized area of the skin.

Guinea Bissau, and Sierra Leone) were incorporated into APOC. A recent study attempted to quantify the health impacts of APOC, and they are impressive [6]. For example, an estimated total of 19 million disability-adjusted life years (DALYs) have been averted, and this represents an 80% reduction in DALY loss for APOC countries. Unfortunately, these improvements may not be permanent because resurgence of transmission and disease may occur if CDTI is discontinued prematurely. This could either be due to a lack of logistical support to continue CDTI or because diagnostic tests prematurely indicated that infection prevalence was low enough to stop CDTI. While recent studies suggest that local elimination of onchocerciasis has been achieved in some areas after a minimum of 10 years of CDTI [4,5,7–11], programs for onchocerciasis elimination based on annual CDTI will require active maintenance for many years to come in most African countries.

Until recently, APOC activities were focused on hyper- and mesoendemic areas where disease rates were highest. However, the global health community has recently changed the public

health goal for onchocerciasis from disease control to elimination [11,12]. The switch to elimination will require extending ivermectin coverage into extensive areas in Africa where fewer than 20% of adult men have palpable onchocercal nodules that have not previously been eligible for CDTI. One reason for including hypoendemic areas in the elimination program is that they are not always free of disease [13]. In addition, a recent analysis of pre-control data from Africa suggests that a 20% nodule prevalence in men in untreated areas corresponds to a median Mf prevalence in the general population of almost 35% [14]. Therefore, some areas classified as hypoendemic based on the prevalence of onchocercal nodules in small samples of adults, which was the basis for **rapid epidemiological mapping of onchocerciasis** (REMO), may have fairly high infection rates based on Mf testing.

Diagnostic tests such as nodule palpation and skin-snip surveys for Mf and strategies such as REMO that were useful for identifying priority areas for *O. volvulus* infection prevention activities [15] may not be adequate tools for elimination programs (see below). Therefore, the purpose of this paper is to review available and emerging diagnostic tests for onchocerciasis and to consider how they might be deployed during different stages of onchocerciasis elimination programs.

Diagnostic Test Options

Diagnostic test options for onchocerciasis elimination programs are summarized in Box 1 and in the Key Table (Table 1), and are discussed in more detail below.

Box 1. Diagnostic Options for Onchocerciasis Elimination Programs

(i) Clinical Examination

Clinical examination of individuals for *Onchocerca* nodules, dermatitis, or ocular disease requires special skills, and lacks sufficient sensitivity and specificity for use in elimination programs.

(ii) Skin-Snipping

Microfilariae (Mf) can be detected in superficial skin biopsies by microscopy. This method has excellent specificity, but sensitivity is moderate, and it is reduced after ivermectin treatment. PCR testing of skin snips increases sensitivity, but this is not always feasible for programmatic use. Endemic populations are increasingly refusing skin-snip testing.

(iii) The Diethylcarbamazine (DEC) Patch Test

Topical DEC kills Mf in the skin, which results in papule formation. The test must be read 24–48 h after application, and this reduces feasibility. As with skin snips, the sensitivity of this method is reduced after ivermectin treatment, and the specificity of the test has not been clearly demonstrated [30–33].

(iv) Detection of O. volvulus *larvae in* Simulium *flies*

Molecular xenomonitoring (MX) detects DNA of *O. volvulus* infective larvae in pooled heads of *Simulium* vectors, and this is much more sensitive than dissection followed by microscopy. MX has been used as a useful endpoint measure by OEPA in Latin America and in some studies in Africa [4,5,8,35–37]. However, the implementation of MX is difficult at the national level, and it has a high requirement for laboratory infrastructure, trained personnel, and expensive imported supplies.

(v) Antibody Test

Extensive research has shown that tests for IgG4 antibodies to recombinant antigen Ov-16 are specific and moderately sensitive for infection or heavy exposure to *O. volvulus*. A point of care antibody test for Ov-16 has recently been marketed [51]. This test may be useful for mapping hypoendemic areas and for detecting relatively recent transmission events in children. However, no field studies have been published to date on the performance of this test in different endemic settings.

(vi) Biomarker Detection

A sensitive, specific, and practical test for the presence of living adult worms would be very useful for all stages of onchocerciasis elimination programs. It could also be used to assess the efficacy of new treatments for onchocerciasis. Although this is an area of active research, no such test exists at this time.

Key Table

Table 1. Value of Diagnostic Tools for Different Stages of Onchocerciasis Elimination Programs

			Microfilaria detection				
Test	Clinical examination	DEC[a] Patch test	Skin snip microscopy	DNA detection (e.g. PCR)	Molecular xeno-monitoring	Biomarker test for adult worms[b]	Antibody test
Procedure	Nodule palpation, examine skin, eyes	Observe dermal papules after topical DEC	Skin snips to detect Mf[a]	Detection of Mf DNA in the skin	Detection of parasite DNA in black flies	Adult worm biomarker assay (rapid test or central labs)	Antibody assays (rapid test or central labs)
Sensitivity	Low	Medium	Medium	High	High	Unknown	Moderate to high
Specificity	Medium	Unknown	High	High	High	Unknown	High
Advantages	Non-invasive REMO[a] is sensitive for detecting hyper- and meso-endemic areas	Non-invasive detection of Mf	Low-tech test. Provides Mf counts	Very sensitive. Can test pooled samples	Highly sensitive and specific	Adult worm marker, not affected by recent ivermectin treatment	Not affected by recent ivermectin, Useful as an exposure marker in children
Disadvantages	Low sensitivity for hypo-endemic areas	Test is read at 24–48 h. Insensitive post-ivermectin	People object to skin snips. Insensitive post-ivermectin.		Difficulties collecting flies.	Area of active research, but no test is currently available	Antibody tests do not distinguish between past and current infections
				High cost/infrastructure requirement for PCR			
Mapping[c]	1[d]	2 (?)	2	2	1	2 (?)	2 (?)
Midcourse monitoring[c]	0	1 (?)	1	1	2	2 (?)	0
Stopping MDA[a,c] **decision**	0	1 (?)	1	1	2	2 (?)	2
Post-MDA surveillance[c]	0	1 (?)	1	1	2	2 (?)	2

[a]Abbreviations: DEC, diethylcarbamazine; Mf, microfilaria; MDA, mass drug administration; REMO, rapid assessment method for onchocerciasis.
[b]Because there is no good antigen test at this time, described test characteristics are based on currently available antigen tests for other filarial infections.
[c]More data are needed on use of all of these tests in onchocerciasis elimination programs. Items with greatest uncertainty are marked with '?'.
[d]The relative value of each test for the different stages is indicated by numerals. 0, not useful; 1, useful; 2, very useful.

Clinical Examination

Clinical signs of onchocerciasis can be detected by ocular examination (measuring visual acuity and visual fields using a slit lamp), by examination of the skin for signs of onchodermatitis, or by investigation of subcutaneous nodules by palpation or ultrasonography. Ocular examination is insensitive for *O. volvulus* infection (many infected people lack ocular involvement), and slit lamp examination requires special expertise and expensive equipment. Some forms of onchodermatitis, such as leopard skin and **sowda**, have moderate to high diagnostic specificity, but they are not sensitive markers for onchocerciasis; many individuals with Mf in skin snips have little or no skin disease.

Nodule palpation has been widely used to map the distribution of onchocerciasis. The subcutaneous nodules (onchocercomata) are sometimes visible and more often palpable, notably adjacent to bony prominences such as the iliac crest, but they also occur in many other areas [16,17]. Because many people with onchocerciasis do not have palpable nodules, this method is not sensitive for ruling out infection in individuals [6,18–21]. APOC used nodule palpation to identify areas where people were at high or moderate risk of developing clinically-apparent disease due to onchocerciasis (REMO). The method used nodule palpation results from 30–50 adult males per village to assign endemicity status. Because nodule prevalence is correlated with Mf prevalence, areas with nodule prevalence of >20% were classified as having meso- or hyperendemic onchocerciasis with Mf prevalence usually >35%, and these areas qualified for CDTI [15,22]. REMO was not designed to detect or subclassify areas with hypoendemic onchocerciasis.

Ultrasonography has been used to detect onchocercomata in humans and in animals [23,24]. Although it is probably more sensitive than palpation for detecting nodules (especially deeper onchocercomata), it is impractical for programmatic use because it requires special equipment and trained personnel.

Detection of Mf in the Skin

Skin Mf can be identified by microscopic examination of skin snips. Skin snips (superficial biopsies weighing 1–2 mg) are typically incubated in saline for 30 minutes and then examined for emergence of Mf. Longer incubation times increase the sensitivity but decrease the practicality of this method [25]. Skin-snip microscopy is more sensitive than clinical examination for detecting active infections. An even higher sensitivity can be achieved when skin snips are analyzed for the presence of parasite DNA by PCR. Several studies have reported results based on amplification and detection of an *O. volvulus*-specific, noncoding 150 bp tandem repeat sequence (O-150) [26–28]. Toe *et al.* [29] showed that the O-150 PCR could also be performed on superficial skin scrapings. Although skin-snip PCR was more sensitive, the less-invasive skin-scratch PCR method was more sensitive than skin-snip microscopy, especially for detecting light infections. However, the detection of dead or partly fragmented larvae in the skin by PCR might have caused some of the discrepancy between the two techniques [30].

Technical advances have improved the feasibility of DNA detection as a practical diagnostic tool. These include simplified methods for detecting amplification products [31–33] and different amplification methods such as **loop-mediated isothermal amplification** (LAMP) assays [34] or real-time PCR [35]. Important potential advantages of real-time PCR are its high throughput and high sensitivity, which should allow testing of large numbers of pooled samples to estimate prevalence in hypoendemic areas. Despite these advances, few national onchocerciasis programs in Africa have the laboratory facilities, funding, or trained personnel required to make detection of *O. volvulus* DNA a practical diagnostic option at this time.

Diethylcarbamazine (DEC) Patch Test

This test indirectly detects Mf in the skin by inducing a localized Mazzotti reaction with topical **DEC** cream in a gauze material that is applied to the skin with an adhesive bandage. Different versions of this test have been evaluated over many years [36–39]. Pruritic papules appear in response to dying Mf 1–2 days after application of the patch. The sensitivity of this method has been reported to be similar to or slightly higher than skin-snip microscopy, but it is less sensitive than DNA detection. DEC patch test results in children aged 3–5 years have been shown to be correlated with the prevalence of onchocerciasis nodules in subjects aged 5 years and above at the same study site [40]. False positive results have been reported from some patients with **loiasis** [41], but Ozoh *et al.* [40] showed that the DEC patch test could be used to assess and follow-up onchocerciasis endemicity levels in areas with coendemic loiasis. Toe *et al.* [38] reported that *Mansonella perstans*-infected individuals did not have positive reactions with the DEC-patch, but no information is available on whether this is also true for the skin-dwelling *Mansonella streptocerca.* Regardless of whether skin snips, patch tests, or PCR are used, these Mf-based tests will have low sensitivity for onchocerciasis in areas where skin Mf prevalence and counts have been reduced by widespread use of ivermectin.

Molecular Xenomonitoring

Parasite DNA can also be detected in *Simulium* vectors. **Molecular xenomonitoring** (MX) has been used to evaluate onchocerciasis transmission dynamics following years of ivermectin distribution in Latin America [7,42–45] and parts of Africa [8,11]. Recent developments related to MX have included development of an isothermal LAMP assay for detection of *O. volvulus* DNA in black flies [34] and trapping methods that can be used to replace human bait for capturing flies [46–48]. It remains to be seen whether these advances will make MX more feasible for evaluating national or regional onchocerciasis elimination programs in Africa. Challenges include the cost of laboratories and supplies for PCR, a shortage of properly trained personnel, and the difficulty of collecting large numbers of human-biting *Simulium* flies (even during peak transmission seasons) to adequately represent vector populations.

Antibody Tests

The development of antibody tests for onchocerciasis with native antigens was hampered by the scarcity of parasite material (adult worms can only be obtained by nodulectomy) and by low specificity [49,50], although this was improved by measuring IgG4 subclass antibodies [51]. Assays based on recombinant *O. volvulus* antigens varied in terms of sensitivity and specificity (Table S1 in the supplementary material online). Several studies have reported increased sensitivity with tests based on antigen combinations [52,28,53–55]. However, antigen combinations can reduce specificity, and these tests were not commercialized.

The most promising recombinant antigen (Ov-16) [56,57] has been used in several assay platforms [57–59]. Ov-16 antibody tests have been reported to have excellent specificity and moderately high sensitivity (75–85% with samples from people with Mf-positive skin snips). Although antibody tests for onchocerciasis (including Ov-16 tests) cannot distinguish between past and current infections, the presence of anti-Ov-16 antibodies in young children provides evidence for recent transmission. Indeed, several studies have shown that Ov-16 is useful for assessing ongoing transmission of onchocerciasis following CDTI in Latin America and Africa [4,5,7,10,60,61]. A rapid format cassette test for IgG4 antibodies to Ov-16 has recently been marketed for use with finger-prick blood [62] (see also http://sites.path.org/dx/ntd/oncho/), and this should increase the feasibility of antibody testing for onchocerciasis elimination programs.

Biomarkers

A biomarker test would have advantages over antibody assays if it was specific for current infection or if it provided an indication of infection intensity. Sensitive tests have been developed

that detect circulating filarial antigens from adult *Dirofilaria immitis* or *Wuchereria bancrofti* in host blood or serum [63–66]. In addition, filarial antigen levels have shown to be related to the number of adult filarial parasites in several host–parasite systems [67–69] and to the number of Mf in the blood or skin [19,70,71]. Some of these assays are also useful for monitoring the success of macrofilaricidal treatment [72–75]. In contrast to this favorable experience, progress in developing antigen tests for onchocerciasis has been slow and uncertain, and this work is summarized in Table S2. Many immunoassays have been described, and one group has described a metabolite of a host protein in urine as a biomarker for infection [76]. Unfortunately, these tests are not practical for field use, and none has passed rigorous testing of sensitivity and specificity. For example, a promising monoclonal antibody-based assay for circulating *O. volvulus* intermediate filament was set aside because of variable sensitivity with samples from different regions and because of crossreactivity with serum samples from people with other filarial infections [77]. In addition to these problems, none of the antigen or biomarker tests has been independently validated, and none are commercially available.

Detection of parasite-derived miRNAs has been suggested as an alternative target for diagnosis of filarial infections. For example, Tritten *et al.* [78] recently reported the presence of circulating miRNAs from *O. volvulus* in human sera. However, detection of miRNAs is technically difficult, and the sensitivity and specificity of this approach have not yet been assessed.

In theory, a practical, sensitive, and specific biomarker test for active *O. volvulus* infection would be very useful in all stages of onchocerciasis elimination programs. Several groups are actively working to develop such a test. In the meantime, the next section will focus on the use of currently-available diagnostic tests for different stages of onchocerciasis elimination programs.

Selection of Diagnostic Tests for Different Phases of Onchocerciasis Elimination Programs

Different diagnostic tests may be required for different phases of onchocerciasis elimination programs [79–81].

Mapping

APOC used REMO mapping to identify hyper- and mesoendemic areas that require CDTI to control onchocerciasis. A different type of mapping will be needed for onchocerciasis elimination programs. Because meso- and hyperendemic areas are already largely known, mapping for elimination programs needs to identify hypoendemic areas that require intervention. Consequently, one cannot consider mapping options without also considering the unresolved issue of inclusion criteria for the onchocerciasis elimination program. For example, some experts have suggested that hypoendemic areas with nodule prevalence that does not exceed 5% by REMO should be excluded from the program because infections in such areas will gradually die out if they are no longer adjacent to areas with higher prevalence. One problem with this hypothesis is that it has not been rigorously tested; one study documented sustained transmission in hypoendemic areas in Cameroon [82]. Furthermore, REMO nodule prevalence surveys are not powered to accurately classify areas as being above or below 5%. This could potentially lead to misclassification of large areas. In addition, because areas with nodule prevalence in the range of 5% may have skin Mf prevalence that exceeds 10% [14], Mf-positive humans (and infected flies) from endemic areas in the periphery of transmission zones could migrate and reintroduce the parasite into areas where onchocerciasis had previously been eliminated if the areas still have vectors and environmental conditions that are favorable for transmission.

Mf detection tests such as skin-snip microscopy or the DEC patch test may be better options than nodule palpation for mapping hypoendemic areas. However, skin-snipping is unpopular in

some areas, and the sensitivity and specificity of the DEC patch test have not been thoroughly verified relative to skin-snip microscopy. In addition, these tests may not be reliable for detecting active infections in populations that have recently received ivermectin irrespective of whether this was for onchocerciasis or for **lymphatic filariasis** (LF). Even if one assumes that Mf detection is feasible for use in areas that have not recently received ivermectin, it is still not clear what minimum Mf prevalence should be used for including areas in the onchocerciasis elimination program. The threshold selected will lead to other considerations of sample size and sampling methods that are beyond the scope of this review. Another option for Mf detection would be skin-snip PCR using pooled snips collected from different individuals [35]. While technically and logistically difficult to employ on a programmatic level, this could be used in specialized laboratories as a medium- or high-throughput method that would also enable archiving of parasite DNA. It would certainly be less expensive than testing individual skin snips.

Entomology-based mapping of hypoendemic areas is theoretically possible, but it may not be feasible for programs because of cost and infrastructure requirements and because crucial background information needed for efficient vector collection may be missing.

Antibody testing may be a better option than REMO or Mf testing for mapping areas with hypoendemic onchocerciasis. Antibody prevalence should not be affected by recent ivermectin treatment, and antibody test results from mapping studies would provide useful baseline data for later assessments of the impact of interventions and for endpoint studies. However, because they have not been extensively used for this purpose to date, further research will be necessary to establish best practices for using antibody tests such as the Ov-16 ELISA and cassette tests as mapping tools. It will be especially important to document how antibody rates in adults and children compare to Mf and nodule rates in areas with differing levels of endemicity. A combined approach might be useful. For example, the Ov-16 antibody test could be used as a screening tool, and Mf testing could be reserved for those with positive antibody tests to assess the Mf reservoir in communities.

Coendemic loiasis is an important challenge for onchocerciasis elimination programs. APOC has used RAP-LOA as a rapid assessment tool that relates the prevalence of key clinical manifestation of loiasis to the level of endemicity of the infection to estimate loiasis rates in populations [83]. The current policy is to provide ivermectin in areas with hyper- or mesoendemic onchocerciasis plus loiasis. Areas with hypoendemic onchocerciasis with low rates of loiasis (RAP-LOA rates <20%) are eligible for ivermectin mass drug administration (MDA), but MDA is not recommended for areas with hypoendemic onchocerciasis that have RAP-LOA rates ≥20%. Improved diagnostic methods are urgently needed for efficiently mapping such coendemic areas.

Midcourse Monitoring and Evaluation

After CDTI has been initiated, it may be useful to perform periodic assessments to determine whether the program is on track or whether additional measures are needed (e.g., raise compliance, increase treatment frequency, or add vector control). Nodule palpation is not useful for this purpose. By contrast, CDTI coverage surveys or Mf surveys performed soon after CDTI can provide useful information on compliance and the impact of ivermectin.

As mentioned above, antibody tests based on Ov-16 have been used to monitor the success of onchocerciasis control programs. Because IgG4 antibodies to Ov-16 and other *O. volvulus* antigens sometimes persist for many years in adults, antibody surveys for interim monitoring should focus on young children to document reductions in antibody prevalence. Note that several years of decreased transmission may be required before antibody prevalence in children decreases significantly.

CDTI Endpoints and Post-CDTI Surveillance.

When CDTI has decreased *O. volvulus* infection and transmission, the question then becomes 'when is it safe to stop?' Premature stopping could result in resumption of transmission, while unnecessary continuation of the intervention wastes precious effort and money. If targets have not yet been validated, it makes sense to test them by stopping the intervention(s) and closely monitoring changes to detect early signs of recrudescence.

Studies from Senegal and Mali indicate that *O. volvulus* transmission was interrupted in several foci after many years of annual or semiannual CDTI [8,11]. The two main indicators used in these studies were Mf prevalence and vector infectivity as determined by PCR of *Simulium* heads. The target for Mf prevalence was <1% in 90% of the sampled villages and <5% in all of the sampled villages. The threshold for fly infectivity was 1/2000 (0.05%). APOC has also used these criteria as targets for stopping CDTI (www.who.int/apoc/oncho_elimination_report_english.pdf). No recrudescence of infection or transmission has been detected in areas that achieved these targets [8]. However, additional studies are needed to further validate these targets. Vector monitoring is discussed further below.

Antibody testing provides a potentially attractive method for endpoint assessment because it can be integrated with serological surveillance of other neglected tropical diseases (NTDs). Other advantages are that it does not require skin snips or the use of human bait to collect representative samples of host-seeking flies. Antibody rates in populations decrease after transmission has been interrupted, but antibodies to Ov-16 sometimes persist in adults for many years [4]. For this reason, testing should focus on children born after transmission has already been significantly reduced or interrupted by CDTI. The Onchocerciasis Elimination Program for the Americas (OEPA) and pilot projects in Africa have used 0.1% as a target prevalence for antibodies to Ov-16 in children [4,5,7,10,61]. This may have been based on initial guidelines for target **antigenemia** prevalence in children that were proposed by the Global Program to Eliminate Lymphatic Filariasis (GPELF) (http://whqlibdoc.who.int/hq/2005/who_cds_cpe_cee_2005.50.pdf). However, GPELF soon found that this target was too stringent and also not feasible for widespread implementation. Revised GPELF guidelines call for systematic sampling of children in large evaluation units to show with 95% confidence that infection prevalence is less than 2% (http://whqlibdoc.who.int/publications/2011/9789241501484_eng.pdf); this target prevalence may also be reasonable for Ov-16 antibodies in children. However, sampling protocols (age range, evaluation units, etc.) have not yet been developed or adequately tested for this purpose in Africa.

Molecular xenomonitoring has been used in several countries as an alternative to dissection to detect infections in *Simulium* flies [7,11,42–44]. However, questions remain regarding the proper target (estimated infectivity) for this method and its feasibility for assessing programs across Africa. Recent publications have reported progress on methods for collecting host seeking flies [46–48], but this does not solve the problems of restricted times for fly collection in areas with seasonal transmission of onchocerciasis and the high requirements for skilled personnel and expensive laboratory infrastructure. The number of insects required for vector monitoring might be lower if the strategy were changed to detect any stage of infection in the flies (as a measure of the persisting reservoir of Mf in humans in the area) instead of the current focus on vector infectivity.

Next Steps and Research Priorities

At this early stage of the onchocerciasis elimination program in Africa, a top priority will be for onchocerciasis stakeholders to develop consensus definitions for onchocerciasis endemicity, elimination, and recrudescence. Agreement on these points will inform decisions regarding mapping, inclusion criteria, and rational endpoint targets for parameters that can be practically measured. Apart from the issue of definitions, several research priorities are mentioned in the Outstanding Questions Box.

Additional research is needed to expand the evidence base regarding metrics for planning and assessing onchocerciasis elimination programs in Africa. Because mapping of hypoendemic areas is a high priority, operational research is needed to compare results of skin-snip microscopy for Mf, pool screen skin-snip PCR, the DEC patch test, and Ov-16 antibody testing in hypoendemic areas with REMO nodule prevalence between 1% and 20%. When the issue of inclusion criteria has been settled, the next step will be to design and test sampling protocols for mapping hypoendemic areas using the most informative test(s).

Field research should also be performed in several areas with low-level persistence of onchocerciasis following multiple rounds of CDTI. These studies should compare different sampling protocols and assessment tools (skin-snip microscopy for Mf, the DEC patch test, the Ov-16 antibody test, and two tests of *Simulium* infectivity, namely dissection and MX). It would also be interesting to compare *O. volvulus* incidence rates and infectivity in *Simulium* vectors in such areas. Results from these studies would help to establish and validate targets and sampling protocols that can be used for CDTI stopping decisions and for post-CDTI surveillance. Of course it is possible that no single test will be sufficient to verify onchocerciasis elimination. It should be noted that several studies have shown that antibody testing of school age children and MX are more sensitive than Mf or antigen testing for detecting persistence of LF following MDA [84–86], and this is likely to be true for onchocerciasis as well.

Many areas in Africa are coendemic for onchocerciasis and LF, and APOC has recently outlined a plan for integrating elimination activities for these infections in a new entity after its closure (www.who.int/apoc/en_apoc_strategic_plan_2013_ok.pdf). Integrated surveillance for LF and onchocerciasis is also essential in such areas because decisions to stop MDA need to consider the current status of both infections. Recent publications have raised the issue of integrated surveillance for a broader range of NTDs [80,87–89]. The move toward integration of NTD control and elimination programs should lead to new tools and strategies for integrating surveillance activities.

Finally, it should be mentioned that recommendations for mapping and surveillance of onchocerciasis could be very different if we had a sensitive, specific, and operationally-feasible biomarker assay for adult worm infection. A biomarker assay would also be very helpful for use in clinical trials of new treatments for onchocerciasis. Several groups are working on this problem, and it remains a research priority.

Outstanding Questions

What is the relative value of different diagnostic tests and sampling methods for mapping areas with hypoendemic onchocerciasis?

What is the relative value of different diagnostic tests and sampling methods for detecting persistent onchocerciasis following years of mass drug administration?

What tests and strategies are most appropriate for identifying areas with hypoendemic onchocerciasis where CDTI may not be safe because of coendemic loiasis.

What are appropriate targets for onchocerciasis elimination in different endemic areas, and how should recrudescence be defined?

How can surveillance for onchocerciasis and other neglected tropical diseases be integrated?

Can a sensitive and specific onchocerciasis biomarker test be developed in the near future?

Can results obtained before and after CDTI with different (new) diagnostic tests be used to refine and further validate onchocerciasis transmission models?

Concluding Remarks

Diagnostic testing may be as important as CDTI for the ultimate success of onchocerciasis elimination programs. Tools such as REMO, Mf detection, and dissection of flies that were useful for control programs are not optimal for managing elimination programs. Different tests and testing strategies are needed for mapping hypoendemic areas and for knowing when to stop interventions. Operational research should focus on collecting data to help define the best diagnostic tools and best practices for use during different stages of onchocerciasis elimination programs.

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