Acta Tropica 146 (2015) 114–118

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Conventional parasitology and DNA-based diagnostic methods for onchocerciasis elimination programmes

Melanie M. Lloyd[a,*], Rebecca Gilbert[a], Nathalie Tebao Taha[b], Gary J. Weil[a], Aboulaye Meite[b], Ilunga M.M. Kouakou[b], Peter U. Fischer[a]

[a] Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA
[b] Ministry of Health and Social Welfare of Côte d'Ivoire, Abidjan, Cote d'Ivoire

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 16 March 2015
Accepted 17 March 2015
Available online 25 March 2015

Keywords:
Onchocerciasis
Diagnosis
qPCR
Molecular diagnostics

ABSTRACT

Commonly used methods for diagnosing *Onchocerca volvulus* infections (microscopic detection of microfilariae in skin snips and nodule palpation) are insensitive. Improved methods are needed for monitoring and evaluation of onchocerciasis elimination programmes and for clinical diagnosis of individual patients. A sensitive probe-based qPCR assay was developed for detecting *O. volvulus* DNA, and this was tested with samples collected from an endemic area in eastern Côte d'Ivoire. The new test was evaluated with dried skin snip pairs from 369 subjects and compared to routine skin snip microscopy and nodule palpation results from the same individuals. Onchocerciasis prevalence for these samples by qPCR, skin snip microscopy, and nodule palpation were 56.9%, 26.0%, and 37.9%, respectively. Furthermore, the combination of all three tests produced an infection prevalence of 72.9%, which was significantly higher than 53.1% detected by microscopy plus nodule palpation without qPCR. However, the qPCR assay was negative for 54 of 229 individuals with palpable nodules. qPCR could be a useful tool for detecting residual *O. volvulus* infections in human populations as prevalence decreases in areas following community-directed treatment with ivermectin.

1. Introduction

Onchocerciasis (or 'river blindness') is caused by the filarial parasite *Onchocerca volvulus*. An estimated 37 million people are infected by the parasite (mostly in sub-Saharan Africa), and more than 100 million are at risk of acquiring the infection (African Programme for Onchocerciasis Control, 2010; Winthrop et al., 2011). Onchocerciasis is classified by the World Health Organization as a neglected tropical disease (NTD). Following the success of the Onchocerciasis Elimination Program for the Americas (OEPA) and case studies showing feasibility of onchocerciasis elimination within the African Programme for Onchocerciasis Control (APOC), the goal of intervention for onchocerciasis has shifted from disease control to global elimination (Cupp et al., 2011; Diawara et al., 2009; Mackenzie et al., 2012; Traore et al., 2012). The elimination effort is largely based on repeated cycles of mass administration of ivermectin (community-directed treatment with ivermectin, or CDTI). Until recently, CDTI was restricted to areas with hyper- and mesoendemic onchocerciasis prevalence as assessed by rapid epidemiological mapping of onchocerciasis (REMO) that is based on the onchocercal nodule rate in adult males as assessed by palpation (Noma et al., 2002; World Health Organization, 1998). However, the new elimination goal requires CDTI in areas with lower infection prevalence that may not be efficiently detected by nodule palpation. In addition, ivermectin decreases the sensitivity of microscopy for detecting MF in skin snips. Therefore, new strategies for onchocerciasis diagnosis are needed.

Available diagnostic tools for onchocerciasis include microscopic detection of microfilariae (MF) in skin snips, indirect detection of MF with the diethylcarbamazine (DEC) patch test, detection of antibodies to onchocercal antigens, or detection of *O. volvulus* DNA in skin snips by PCR (Udall, 2007; Winthrop et al., 2011). MF in the skin are necessary for continued transmission by black fly vectors, and microscopic detection of MF in skin snips is a useful epidemiological tool. However, it is generally accepted that this test is less sensitive than molecular detection of parasite DNA in skin snips (Fink et al., 2011; Fischer et al., 1996; Zimmerman et al., 1994). Previously developed DNA tests detect an *O. volvulus*-specific 150 bp repeated DNA segment called O-150 (Meredith et al., 1989) by conventional or quantitative PCR.

* Corresponding author at: Infectious Diseases Division, Washington University School of Medicine, 4444 Forest Park, St. Louis, MO 63108, USA.
Tel.: +1 314 747 5198; fax: +1 314 454 5293.
E-mail address: mlloyd@dom.wustl.edu (M.M. Lloyd).

http://dx.doi.org/10.1016/j.actatropica.2015.03.019

The aims of this study were to develop an improved method for detecting *O. volvulus* DNA and to compare results obtained with this test with those obtained by conventional skin snip microscopy and nodule palpation tests.

2. Methods

2.1. Sample collection

Skin snips for this study were taken as part of a larger, community wide study of individuals living in the Akoupé district of southeastern Côte d'Ivoire in March 2014. This area had received some CDTI in the past, but not for at least 20 months prior to our study. Samples were collected under human studies protocols approved by institutional review boards (IRBs) at Washington University School of Medicine and at the Université Félix Houphouët-Boigny, Côte d'Ivoire. All adult participants in the study provided written informed consent; consent from at least one parent plus assent was required for children to participate in the study. Two tests were performed for detection of onchocerciasis in the field. The first was manual palpation for subcutaneous nodules with special attention paid to areas over the iliac crest, skull, limbs, abdomen, and chest wall. The second test was microscopic detection of MF in skin snips. Briefly, one skin snip was taken from each iliac crest with a Walser punch. Snips were placed in 100 μL phosphate buffered saline (PBS) for 24 h in flat-bottomed 96 well microtiter plates and emerging MF were counted for 24 h. After removal of PBS and MF, skin snips were weighed (average weight, 1 mg per snip) and dried for 24 h in open Eppendorf tubes. It should be noted that the skin snip samples were collected after the emergence of MF and the emerged MF were not included in the sample from which DNA was extracted. While there is a possibility this would decrease the number of individuals identified as positive by qPCR, it was important to directly compare the qPCR result with the results from the microscopic detection of MF. Furthermore, as the sample collecting for this study was part of a larger study, it was important for the sample collection methods to be fast and easy, and returning emerged MF to snips for later DNA testing was not practical.

Tubes were shipped to St. Louis and stored at room temperature until DNA was extracted. DNA was extracted from both skin snips from each subject in one extraction using the OMEGA E.Z.N.A. tissue DNA kit (Norcross, GA) according to the kit instructions. The quantity and quality of extracted DNA was assessed with a NanoDrop Spectrophotometer (ND-1000, Thermo Scientific, Waltham, MA).

2.2. A quantitative PCR assay for detecting O-150 DNA

A TaqMan qPCR assay was designed according to MIQE guidelines (Bustin et al., 2009) to detect the *O. volvulus* O-150 DNA sequence (accession number J04659). The O-150 sequence is a tandem repeat region with a variable number of similar but not always identical repeats; there are more than 4000 copies of this sequence per haploid genome of *O. volvulus* (Meredith et al., 1989). While there is some conservation of this sequence within the genus *Onchocerca*, PCR of this region combined with southern-blot hybridization differentiates between closely related *O. volvulus* and *Onchocerca ochengi*, *Onchocerca dukei*, *Onchocerca armillata*, and *Onchocerca gutturosa* (Fischer et al., 1996). Primer Express 3.0.1 (Applied Biosystems Thermo Scientific, Waltham, MA) was used to design a primer probe assay that is specific to the *O. volvulus* O-150 region. The assay was primarily designed for use in humans that are not infected with other *Onchocerca* species, but we did check the similarity of our primers/probe with the homologous sequences of *O. ochengi* and *Onchocerca* sp. 'Siisa', because these species sometimes share the same blackfly vectors with *O. volvulus*. The maximum degree of identity of the concatenated primers/probe was 91% and 85% for *O. ochengi* (LL518415.1) and *Onchocerca* sp. 'Siisa' (DQ523790.1), respectively. Primers were purchased from IDT (Coralville, IA). Primer sequences were as follows: F 5′-TCGCCGTGTAAATGTGGAA-3′ R 5′-GATTAGGGTCATAGGTCATCAGTT-3′, and the probe sequence was 5′-GGACCCAATTCGAATGTATGTACCCGT-3′. The probe had a 5′ 6-FAM modification and dual ZEN – 3′ Iowa Black FQ quencher. PCR reactions were performed with an Applied Biosystems QuantStudio 6 instrument (Thermo Scientific, Waltham, MA), and the results were analysed using QuantStudio 6 and 7 Flex Software 2013. PCR reaction mixtures (10 μL total volume) included 1X TaqMan Fast Advanced Master Mix (Life Technologies, Thermo Scientific), 300 nM primers, 250 nM probe, and 2 μL template DNA isolated from skin snips. The concentration of DNA isolated from skin snips ranged from 4 to 65 ng/μL. Cycling parameters included a pre-PCR read (60 °C for 30 s), a hold (95 °C for 20 s), the PCR read stage (40 cycles: 1 s at 95 °C and 20 s at 57 °C), and the post-PCR read stage (30 s at 60 °C). A standard curve was developed using serial dilutions of DNA extracted from adult *O. volvulus* worms to test the sensitivity of the assay. Each DNA sample was tested in duplicate. The qPCR assay was scored as positive if the amplification signal exceeded threshold fluorescence values (automatically determined by the software) in fewer than 40 amplification cycles ($C_q < 40$). Samples were retested if different results were obtained in duplicate wells. Individuals were considered to have positive *O. volvulus* PCR results if two or more of four qPCR reactions were positive. Specificity was tested with DNA samples extracted from nine skin snips from two individuals that had never been exposed to *O. volvulus* and with DNA extracted from adult *O. ochengi* worms and vectors infected with *Onchocerca* sp. 'Siisa'. Furthermore, each 96-well plate (MicroAmp fast optical 96-well reaction plate, Applied Biosystems) included 'no template' control wells that included water in place of DNA template. Most samples with C_q values between 38 and 40 in only one well were not confirmed as positive after repeat testing.

2.3. Statistical analysis

A test of equal proportions was performed to compare results obtained with diagnostic tests on the number of individuals with positive results for the following tests and combinations: qPCR, microscopic detection of MF, nodule palpation, microscopic detection of MF *or* nodule palpation, and 'any test positive'. Post hoc pairwise comparisons of test results were also performed. A linear model (LM) was performed to identify whether the number of microscopically detected MF was a significant predicting factor for the average C_q value obtained with duplicate skin snips. The number of MF was (log + 1) transformed to normalize the data. Statistical tests were performed with open access R software, version 3.1.1 (R Core Team, 2013).

2.4. Conventional PCR assay

A conventional PCR assay was also designed in order to compare the sensitivity of qPCR with conventional PCR. To test the limit of detection of the conventional PCR, a standard curve was performed using the same serial dilutions used with the qPCR standard curve. To compare the sensitivity of conventional and qPCR, 56 DNA extractions were tested by both methods. 25 μL reactions included 21 μL Invitrogen PCR SuperMix High Fidelity (Thermo Scientific) with 200 nM primers (same forward and reverse primers used in the real-time PCR assay), and 2 μL DNA template. PCR was performed using a Bio-Rad MyCycler Thermal Cycler (Hercules, CA). The cycling programme included a 2 min hold at 94 °C; 40

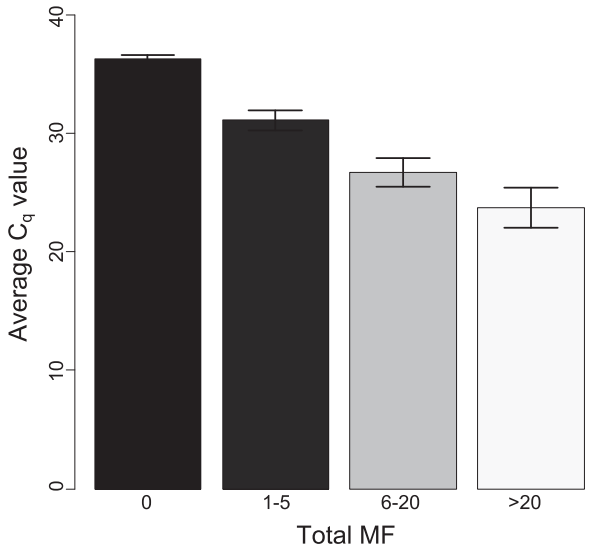

Fig. 1. *Onchocerca volvulus* DNA levels were inversely related to skin microfiladermia counts. The figure shows mean C_q values (±SE) obtained by qPCR with skin snips from people with differing numbers of skin microfiladermia (total MF in two skin snips).

amplification cycles (30 s at 94 °C, 30 s at 55 °C, and 20 s at 68 °C); and a final hold of 7 min at 72 °C. PCR products were separated on a 2% agarose gel and scored as positive or negative based on the presence of a visible band at 150 bp. DNA in gels was visualized using the EZ-vision DNA dye (Amresco, Solon, OH). The results of the conventional PCR assay were compared to results obtained by qPCR, microscopic detection of MF, and nodule palpation using a test of equal proportions.

3. Results

3.1. Quantitative PCR assay to detect O-150

The qPCR assay was assessed with serial dilutions of DNA extracted from adult *O. volvulus* worms. The linear equation of the curve was $y = -3.67x + 16.13$ ($r^2 = 0.996$) and the efficiency was 87.3%. The limit of detection for template DNA was assessed by testing samples with template in the range of 1 ng/μL and 1 fg/μL (thus 2 ng to 2 fg of template DNA in a 10 μL qPCR reaction). The limit of detection was 10 fg/μL, and the mean C_q value for duplicate wells with this amount of template was 34.99 (SD 0.72).

qPCR was performed with DNA samples extracted from skin snip pairs from 369 subjects. Duplicate C_q results matched for 310 DNA samples. The other 59 samples were retested and scored as described in Section 2. All nine skin snips from two individuals not exposed to *O. volvulus* were negative by qPCR as were all 'no template' control reactions and reactions where with template DNA extracted from *O. ochengi* or *Onchocerca* sp. 'Siisa'. The number of microscopically detected MF in skin snips was a highly significant factor in the linear model which compared differences in C_q value ($P < 0.0001$), and C_q values were inversely related to MF number. Therefore, the O-150 qPCR assay provides a semi-quantitative estimate of skin MF counts (Fig. 1).

3.2. Comparison of onchocerciasis test results obtained by different methods

qPCR, nodule, and skin snip microscopy results for 369 individuals are presented in Table 1. The qPCR assay detected significantly more positives than either microscopic detection of MF or nodule palpation alone. Indeed, the qPCR assay detected more positives than the combination of skin snip microscopy plus nodule palpation, but this difference was not statistically significant.

Table 1
Comparison of onchocerciasis test results obtained by qPCR, microscopic detection of microfilariae (MF) in skin snips, and nodule palpation for 369 individuals in the Akoupé district, Côte d'Ivoire.

Method	Positive	Negative	% Pos
qPCR	210	159	56.91
Skin snip MF	96	273	26.00*
Nodule palpation	140	229	37.94*
Skin snip MF or nodules	196	173	53.12
Any test positive	269	100	72.90*

* Rates that are significantly different from the % positive by qPCR ($P < 0.05$, determined by a test of equal proportions).

Table 2
Cross tabulation of test results obtained by qPCR, microscopic detection of microfilariae (MF) in skin snips, and nodule palpation for 369 individuals in the Akoupé district, Côte d'Ivoire.

	Nodule positive		Nodule negative		Total
	MF positive	MF negative	MF positive	MF negative	
qPCR positive	38	48	51	73	210
qPCR negative	2	52	5	100	159
Total	40	100	56	173	369

qPCR results were positive for 137 of 196 (69.9%) individuals that were positive by either microscopic detection of MF or nodule palpation. Of the 59 individuals positive by a conventional test and negative by qPCR, 52 were positive by nodule palpation but MF negative by microscopy and thus likely did not have MF in the skin. The average number of nodules (3.04 nodules) in individuals positive by nodule palpation but negative by qPCR ($N = 54$) was significantly lower than the average number of nodules (4.19 nodules) in individuals positive by both nodule palpation and qPCR ($N = 86$, $P = 0.01$). The maximum number of nodules in the individuals with negative qPCR results was 9 nodules per person while the maximum number of nodules in individuals with positive qPCR results was 12 nodules. Neither the average age nor the proportion of males and females was different between these two groups. Eighty-nine of 96 (92.1%) people positive for MF by skin snip microscopy and 86 of 140 (61.4%) people with palpable nodules had positive skin qPCR results (Table 2). Of the seven people positive by microscopic detection of MF in skin snips and negative by qPCR, the total number of MF detected by microscopy in two skin snips was one for five of the individuals and three and six for the other two. Forty-eight of 100 (48%) people with nodules but negative skin snip microscopy had positive qPCR results.

3.3. Comparison of qPCR with conventional PCR for detecting O-150 in skin snips

The performance of conventional PCR and qPCR was also compared. The limit of detection of the conventional PCR assay was 1 pg/μL. Fifty-six skin snip DNA extractions were randomly selected for testing by conventional PCR, and results are shown in Table 3. qPCR detected more positives than conventional PCR, though not significantly more. However, both PCR assays identified significantly more positive individuals than the combination of microscopic detection of MF and nodule palpation for this subset of study subjects.

4. Discussion

This study reports the first use of qPCR for detecting *O. volvulus* DNA in dried, field collected skip snips from an onchocerciasis

Table 3
Comparison of onchocerciasis test results obtained by qPCR, conventional PCR, microscopic detection of MF in skin snips, and nodule palpation for 56 randomly selected subjects in Akoupé district, Côte d'Ivoire.

Method	Positive	Negative	% Pos
qPCR	26	30	46.43
Conventional PCR	19	37	33.93
Microscopy or nodules	14	42	25.00*

* Rates that are significantly different from the % positive by qPCR ($P < 0.05$, determined by a test of equal proportions).

endemic area. Results presented show that the qPCR assay is highly sensitive and quantitative. qPCR detected more positive individuals than conventional PCR (though not significant) and had 100-fold lower limit of detection. qPCR also has other advantages over conventional PCR: it is faster (one run on the QuantStudio 6 takes approximately 30 min while a comparable PCR run on the BioRad MyCycler takes 2 h and also requires running an agarose gel), it provides more objective results (qPCR results are scored based on automated measurement of fluorescence while results from conventional PCR are scored based on visual detection of band on an agarose gel), and it is less prone to contamination (qPCR does not require running PCR products on an agarose gel, and PCR reactions can be thrown away immediately following amplification).

Our results also show that qPCR is more sensitive than skin snip microscopy or nodule palpation for detecting onchocerciasis and it is at least as sensitive as the combination of these two traditional diagnostic methods. Both of the conventional parasitological tests performed poorly on their own. For example, 129 of 229 individuals who did not have palpable nodules (56.3%) had positive qPCR or skin snip microscopy results. Likewise, 173 of 273 individuals (63.4%) with negative skin snip microscopy results had palpable nodules or qPCR results. Thus, combining positive results from all three tests significantly increased the prevalence of positivity in the sampled population (Table 1). However, 52 individuals had palpable nodules but were MF negative by both microscopy and qPCR and likely did not have MF in the skin. This may be due to the occurrence of infertile *O. volvulus* females in onchocercomas after repeated cycles of mass administration of ivermectin.

While it would be difficult to justify the cost of performing qPCR on entire populations, it should be feasible to test representative samples of populations or sentinel groups. qPCR could be used for mapping endemic areas and it could be especially useful for assessing areas for low level persistence of infection following mass drug administration. The high sensitivity of qPCR means that it should be possible to detect a single positive sample in a pool of samples from at least 10 people and this would greatly reduce costs. Pool screen PCR has been widely used in onchocerciasis-endemic regions for estimating filarial infection prevalence in insect vectors (Katholi et al., 1995) and PCR with pooled human blood samples has been used estimate filariasis infection prevalence in Indonesia (Supali et al., 2006). In the context of onchocerciasis elimination programmes, qPCR (alone or together with skin snip microscopy) may be especially useful for assessing the persistence of onchocerciasis in transmission zones or implementation units prior to termination of CDTI. qPCR is an easy addition to the current field assessment activities, because it uses skin snips that are already collected for microscopic detection of MF. It could either be used to supplement skin snip microscopy, or it could be used instead of microscopy in some settings.

The qPCR test relies on skin snipping which often causes pain and has a potential for transmission of blood borne infections. The WHO has encouraged use of the less invasive DEC patch test which indirectly detects MF by inducing a strong inflammatory response caused by DEC when it comes in contact with MF (Boatin et al., 2002). However, there were two major reasons why the DEC patch test was not included in this study. First, there is no standardized commercial version of the test available, and we questioned the value of using a homemade test in our study. Second, the patch test is read after 24 or 48 h; this is an important limitation of the patch test, and timed follow-up visits were not feasible in this study. A new rapid format antibody test has recently been marketed that detects IgG4 antibodies to recombinant antigen OV-16 (Golden et al., 2013). However test does not differentiate between pre-patent, present and past infections, because antibodies persist for years after infections are cleared. One strategy that might prove useful would be to use the rapid format OV-16 test for screening and the qPCR assay reported here for verification of active infection, since qPCR is more sensitive than MF detection by microscopy.

Interestingly, 100 subjects in this study had palpable nodules with negative skin snip microscopy results, and 52% of these people had negative qPCR results. There are several possible explanations for these results, and they are not mutually exclusive. First, nodule palpation can sometimes produce false positive results due to misclassification of lymph nodes, lipomas, or cysticeri. Second, prior ivermectin treatment may have cleared MF from the skin without eliminating onchocercal nodules in some cases. However, we believe that ivermectin is not likely to have been an important factor, because the area had not received CDTI for at least 20 months prior to our study. The third explanation is that MF and parasite DNA may have been simply absent from the two snips taken from some individuals with very light infections. Finally, some subjects with nodules may have had old infections with no remaining reproductively active female worms.

Looking beyond the particulars of this study, it is important to note that qPCR and skin snip microscopy both depend on the presence of *O. volvulus* MF in the skin. qPCR is clearly more sensitive for detecting MF than skin snip microscopy, and it can be performed on 'recycled' specimens. qPCR should be more sensitive than skin snip microscopy for determining whether MF that can be transmitted by blackfly vectors are still present in humans prior to discontinuation of CDTI. Although ivermectin has little permanent effect on female adult worms, the drug clears MF from the skin for a period of months. For this reason, if qPCR is used to verify the absence of MF in sentinel populations, it is important to delay sample collection for a period of months after the last ivermectin distribution to provide time for MF from surviving adult worms to repopulate the skin.

In conclusion, this study has shown that qPCR is more sensitive than skin snip microscopy or nodule palpation for detecting onchocerciasis in individuals and in populations. We believe that qPCR (alone or together with other methods) has the potential to be a useful tool for different phases of onchocerciasis elimination programmes including mapping of endemic areas, monitoring progress, and verification of elimination.

Acknowledgements

We would like to acknowledge Dr. Olivier Kouadio and the Centre Suisse de Recherches Scientifiques and the Medical Assistance Programmes (MAP) Abidjan, Côte d'Ivoire, for logistical support. This study was funded in part by a grant from the Bill & Melinda Gates Foundation (GH5342, the DOLF project). The findings and conclusions in this article do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation.

References

African Programme for Onchocerciasis Control, 2010. Report of the sixth meeting of national task forces, October 2009. In: Weekly Epidemiological Record 85., pp. 23–28.

Boatin, B., Toé, L., Alley, E., Nagelkerke, N., Borsboom, G., Habbema, J., 2002. Detection of *Onchocerca volvulus* infection in low prevalence areas: a comparison of three diagnostic methods. Parasitology 125, 545–552.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622.

Cupp, E., Sauerbrey, M., Richards, F., 2011. Elimination of human onchocerciasis: history of progress and current feasibility using ivermectin (Mectizan®) monotherapy. Acta Trop. 120, S100–S108.

Diawara, L., Traoré, M.O., Badji, A., Bissan, Y., Doumbia, K., Goita, S.F., Konaté, L., Mounkoro, K., Sarr, M.D., Seck, A.F., 2009. Feasibility of onchocerciasis elimination with ivermectin treatment in endemic foci in Africa: first evidence from studies in Mali and Senegal. PLoS Neglect. Trop. Dis. 3, e497.

Fink, D.L., Fahle, G.A., Fischer, S., Fedorko, D.F., Nutman, T.B., 2011. Toward molecular parasitologic diagnosis: enhanced diagnostic sensitivity for filarial infections in mobile populations. J. Clin. Microbiol. 49, 42–47.

Fischer, P., Büttner, D., Rubaale, T., Meredith, S., 1996. Sensitivity of a polymerase chain reaction-based assay to detect *Onchocerca volvulus* DNA in skin biopsies. Parasitol. Res. 82, 395–401.

Golden, A., Steel, C., Yokobe, L., Jackson, E., Barney, R., Kubofcik, J., Peck, R., Unnasch, T.R., Nutman, T.B., de los Santos, T., 2013. Extended result reading window in lateral flow tests detecting exposure to *Onchocerca volvulus*: a new technology to improve epidemiological surveillance tools. PLoS One 8, e69231.

Katholi, C.R., Toé, L., Merriweather, A., Unnasch, T.R., 1995. Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies. J. Infect. Dis. 172, 1414–1417.

Mackenzie, C.D., Homeida, M.M., Hopkins, A.D., Lawrence, J.C., 2012. Elimination of onchocerciasis from Africa: possible? Trends Parasitol. 28, 16–22.

Meredith, S.E., Unnasch, T.R., Karam, M., Piessens, W.F., Wirth, D.F., 1989. Cloning and characterization of an *Onchocerca volvulus* specific DNA sequence. Mol. Biochem. Parasit. 36, 1–10.

Noma, M., Nwoke, B., Nutall, I., Tambala, P., Enyong, P., Namsenmo, A., Remme, J., Amazigo, U., Kale, O., Seketeli, A., 2002. Rapid epidemiological mapping of onchocerciasis (REMO): its application by the African Programme for Onchocerciasis Control (APOC). Pathog. Global Health 96, S29–S39.

R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Supali, T., Ismid, I.S., Wibowo, H., Djuardi, Y., Majawati, E., Ginanjar, P., Fischer, P., 2006. Estimation of the prevalence of lymphatic filariasis by a pool screen PCR assay using blood spots collected on filter paper. Trans. R. Soc. Trop. Med. Hyg. 100, 753–759.

Traore, M.O., Sarr, M.D., Badji, A., Bissan, Y., Diawara, L., Doumbia, K., Goita, S.F., Konate, L., Mounkoro, K., Seck, A.F., 2012. Proof-of-principle of onchocerciasis elimination with ivermectin treatment in endemic foci in Africa: final results of a study in Mali and Senegal. PLoS Neglect. Trop. Dis. 6, e1825.

Udall, D.N., 2007. Recent updates on onchocerciasis: diagnosis and treatment. Clin. Infect. Dis. 44, 53–60.

Winthrop, K.L., Furtado, J.M., Silva, J.C., Resnikoff, S., Lansingh, V.C., 2011. River blindness: an old disease on the brink of elimination and control. J. Global Infect. Dis. 3, 151.

World Health Organization, 1998. Guidelines for Analysis of REMO Data using GIS. WHO, Geneva.

Zimmerman, P.A., Guderian, R.H., Aruajo, E., Elson, L., Phadke, P., Kubofcik, J., Nutman, T.B., 1994. Polymerase chain reaction-based diagnosis of *Onchocerca volvulus* infection: improved detection of patients with onchocerciasis. J. Infect. Dis. 169, 686–689.