Characterizing Reactivity to *Onchocerca volvulus* Antigens in Multiplex Bead Assays

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Abstract. Multiplex bead assays (MBAs) may provide a powerful integrated tool for monitoring, evaluation, and post-elimination surveillance of onchocerciasis and co-endemic diseases; however, the specificity and sensitivity of *Onchocerca volvulus* antigens have not been characterized within this context. An MBA was developed to evaluate three antigens (OV-16, OV-17, and OV-33) for onchocerciasis. Receiver operating characteristics (ROC) analyses were used to characterize antigen performance using a panel of 610 specimens: 109 *O. volvulus*-positive specimens, 426 non-onchocerciasis controls with filarial and other confirmed parasitic infection, and 75 sera from patients with no other parasitic infection. The IgG and IgG4 assays for OV-16 demonstrated sensitivities of 95.4% and 96.3%, and specificities of 99.4% and 99.8%, respectively. The OV-17 IgG and IgG4 assays had sensitivities of 86.2% and 76.1% and specificities of 79.2% and 82.8%. For OV-33, the IgG and IgG4 assays had sensitivities of 90.8% and 96.3%, and specificities of 96.8% and 98.6%. The OV-16 IgG4-based MBA had the best assay characteristics, followed by OV-33 IgG4. The OV-16 IgG4 assay would be useful for monitoring and evaluation using the MBA platform. Further evaluations are needed to review the potential use of OV-33 as a confirmatory test in the context of program evaluations.

INTRODUCTION

Onchocerciasis, also known as river blindness, is a neglected tropical disease (NTD) caused by the parasitic worm *Onchocerca volvulus*. Recent data from the World Health Organization (WHO) estimate that over 187 million people live at risk for infection by *O. volvulus* primarily in sub-Saharan Africa.¹ Infection can result in visual impairment or blindness, mild to severe dermatitis, and nodules under the skin. Of the over 25 million people estimated to be infected, approximately 300,000 are blind and more than 800,000 suffer visual impairment.² Onchocerciasis is targeted for elimination where feasible in Africa by 2025,³ and the current standard to achieve its elimination is through the mass drug administration (MDA) of ivermectin. This anti-parasitic drug kills microfilariae (MF), a juvenile stage of the parasite, but not the adult worms, which are long lived.^{4,5} Fifteen or more years of MDA may be necessary to interrupt transmission⁶ and laboratory-based evaluations need to be performed before stopping the administration of ivermectin. Sensitive and specific monitoring tools are needed for deciding when MDA can be stopped. The WHO 2016 guidelines for stopping MDA for onchocerciasis require an OV-16 seroprevalence of less than 0.1% in children under 10 years old; currently, this must be measured using an enzyme-linked immunosorbent assay (ELISA).⁷ Much of the evidence for this guideline comes from experience in the Americas and some parts of Africa (i.e., Uganda).^{6,8–14} The new guidelines no longer accept skin snip microscopy for the MDA stopping decision because snips, while useful in hyper-endemic and untreated areas, are affected by ivermectin use and are not sensitive in settings with low transmission, such as those nearing elimination.¹⁵ Detection of anti-OV-16 IgG4 antibodies

by ELISA in children is more sensitive than skin snip assays and sample collection is less invasive.¹⁶

As onchocerciasis may be co-endemic with lymphatic filariasis (LF) and other NTDs, integrated monitoring and evaluation (M and E) and surveillance for these diseases could allow more efficient resource use, increase opportunities for surveillance of NTDs, and achieve a more comprehensive view of disease distributions.^{17–19} Multiplex bead assays (MBAs) may provide a powerful tool for integrating surveillance for multiple NTDs.^{20–24} The MBA can analyze antibody responses to 100 antigens simultaneously from a single specimen, using a double signal mechanism that can discriminate and quantify reactivity.^{22,25–28} The inclusion of multiple antigens in the MBA would allow for simultaneous evaluation of a single specimen for multiple diseases and would allow inclusion of confirmatory tests and internal quality controls.

The objective of this study was to evaluate the sensitivity and specificity of three *O. volvulus* antigens in comparison to skin snip polymerase chain reaction (PCR) using the MBA platform: OV-16, OV-33, and OV-17. The OV-16 antigen localizes to the hypodermis, cuticle, and uterus of MF-producing *O. volvulus*.^{29,30} OV-17 localizes to the hypodermis of adult and larval stages of *O. volvulus*, has homologues in *Onchocerca gibsoni* and *Brugia malayi*, and may be a secretory antigen.³¹ OV-33 localizes to the reproductive organs and muscles of adult stages of *O. volvulus*.^{32,33} It is not a highly conserved structural protein, though there are similar genes in *Brugia malayi* and *Dirofilaria immitis*.^{32,34} A homolog of the OV-33 was previously reported to be sensitive (93%) and specific (96%) for the immunodiagnosis of onchocerciasis.³⁵

METHODS

Human samples. A panel of 610 specimens was assembled for this study: 109 *O. volvulus*-positive specimens (microfilaria positive in skin snips either by microscopy or PCR) and 501 from non-endemic controls (Table 1).

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TABLE 1
Characteristics of the serum specimen panel

Infecting agent	No.	Country of origin	Coinfecting organisms
<i>Onchocerca volvulus</i>	94	Uganda	
	15	Ethiopia	
<i>Wuchereria bancrofti</i>	127	Haiti	
	12	Kenya	
	8	Brazil	<i>Necator americanus</i> (N = 1)
			<i>S. stercoralis</i> (N = 2)
			<i>Ascaris lumbricoides</i> + <i>S. stercoralis</i> (N = 1)
			<i>A. lumbricoides</i> , <i>S. mansoni</i> (N = 1)
			<i>Entamoeba histolytica</i> (N = 1)
			<i>Trichuris</i> spp. (N = 2)
	13	India	
	6	Bangladesh	
	13	Sri Lanka	
	5	Tahiti	
<i>Brugia malayi</i>	3	Sulawesi	
	4	Kalimantan	
<i>Loa loa</i>	21	U.S. expatriates	
<i>Mansonella ozzardi</i>	36	Peru	
<i>Schistosoma mansoni</i>	20	Kenya	
<i>Strongyloides stercoralis</i>	30	Argentina	
	19	U.S. expatriates	
<i>Taenia solium</i>	66	Peru	
<i>Toxocara</i> spp.	15	United States	
<i>Ascaris lumbricoides</i>	6	Brazil	<i>Entamoeba</i> spp. (N = 3)
			<i>H. nana</i> (N = 1)
<i>Hymenolepis nana</i>	7	Brazil	<i>Entamoeba</i> spp. (N = 4)
<i>Entamoeba</i> spp.	15	Brazil	<i>Giardia intestinalis</i> (N = 1)
			<i>Iodamoeba butschlii</i> (N = 4)
Negative	49	United States	
	12	India	
	11	Brazil	
	3	Mali	

Onchocerciasis-positive specimens were collected in a protocol designed to evaluate diagnostic tests for onchocerciasis in the African context. The objective of the protocol was to find infected individuals, rather than define prevalence of infection, so that various diagnostic tests could be assessed. A convenience sample of 1,000 people was taken in study sites in Uganda and Ethiopia (500 people each site). Ivermectin distribution had consistently occurred for less than 3 years in both sites; the last ivermectin distribution occurred 5 months before the study. Background information was collected using a standard questionnaire, two skin skips were taken, and venous blood samples (both serum and ethylenediaminetetraacetic acid preserved) were collected from each participant. Skin snips were evaluated in the field by microscopy after incubation for 24 hours in normal saline and by PCR at CDC laboratories. The protocol was approved by ethical review boards in the United States, Ethiopia, and Uganda. Onchocerca-positive samples used in the MBA described herein were from people who were enrolled in this protocol and who had MF in skin snips by microscopy and onchocercal DNA by PCR.

Negative control sera were selected from sample sets of projects done in areas not endemic for onchocerciasis in the following countries: Haiti, Kenya, Brazil, India, Bangladesh, Sri Lanka, Tahiti, Indonesia, United States, Peru, Argentina, and Mali. Among these onchocerciasis negative sera, 191 were positive for LF, 57 for other filarial infections, 135 for other NTDs, and 43 for an enteric parasite. These sera originated from individuals whose parasitic infections were

confirmed by microscopy or radiologic imaging (e.g., neurocysticercosis). Seventy-five of the onchocerciasis-negative sera were also negative for all of the aforementioned infections (Table 1). All sera were collected under protocols with appropriate ethical clearances.

Antigens. OV-16-GST: This antigen was generated as previously described^{30,36} in the laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases and kindly provided by Thomas Nutman.

OV-33: A 627-bp fragment of the *O. volvulus* 33.3 (OV-33) protein coding sequence was amplified from an adult female worm cDNA library in Lambda Uni-ZAP XR (Filariasis Reagent Resource Center at Smith College, Northampton, MA) with primers previously described by Lucius.³² The DNA was amplified and cloned into Rosetta-gamiB(DE3) *Escherichia coli* (Novagen, Madison, WI). The recombinant GST- and His₆-tagged antigen was purified by GST affinity column chromatography (GE Healthcare, Pittsburgh, PA) as previously described.²¹ It was further purified by dialysis versus 2 L phosphate-buffered saline (PBS) (2× at 4°C), resulting in a 2 mL solution of protein at a concentration of 0.57 mg/mL (BCA microassay, Pierce Biotechnology, Rockford, IL).

OV-17: Similarly, the OV-17 coding sequence (minus the 51 bp 5' sequence encoding the 17 amino acid signal peptide) was PCR amplified from an *O. volvulus* cDNA library³¹ and cloned into HB101 *E. coli* cells (Promega, Madison, WI). The GST-tagged recombinant antigen was then purified by GST affinity column chromatography (GE Healthcare). Dialysis of the glutathione-eluted protein against 2 L of PBS (2× at 4°C) yielded approximately

TABLE 2
IgG and IgG4 assay performance by antigen

	IgG anti-OV-16	IgG4 anti-OV-16	IgG anti-OV-17	IgG4 anti-OV-17	IgG anti-OV-33	IgG4 anti-OV-33
Mean MFI-bg (range)	12,423 (4 to 27,024)	21,733 (-4 to 31,084)	15,657 (62 to 29,787)	13,062 (-3 to 30,104)	23,809 (1,092 to 29,372)	26,522 (2 to 31,806)
<i>Onchocerca volvulus</i> positive specimens						
Mean MFI-bg (range)	24 (-8 to 3,451)	2 (-8 to 48)	109 (-2 to 24,193)	2 (-3 to 28,103)	300 (-2 to 25,031)	4 (-7 to 15,428)
<i>O. volvulus</i> negative specimens						
Positivity threshold (MFI-bg)	586	33	915	603	5,216	67
Specificity (95% CI)	99.4 (96.4 to 100)	99.8 (99.4 to 1.00)	79.2 (69.7 to 95.8)	82.8 (63.9 to 92.2)	96.8 (87.4 to 98.2)	98.6 (95.8 to 99.6)
Sensitivity (95% CI)	95.4 (92.6 to 99.1)	96.3 (92.6 to 99.1)	86.2 (70.6 to 96.3)	76.1 (66.9 to 94.5)	90.8 (87.2 to 99.1)	96.3 (93.6 to 100.0)
Area under curve (95% CI)	0.987 (0.969 to 1.00)	0.984 (0.957 to 1.00)	0.912 (0.884 to 0.941)	0.879 (0.842 to 0.916)	0.984 (0.976 to 0.992)	0.991 (0.979 to 1.00)
True negatives	497	500	397	415	437	494
True positives	105	105	94	83	95	105
False negatives	4	4	15	26	1	4
False positives	4	1	104	86	9	7

CI = confidence intervals; bg = background; MFI = mean fluorescent intensity.

3 mL of protein solution at a concentration of 2.6 mg/mL (BCA microassay, Pierce Biotechnology).

Bead coupling. SeroMap beads (Luminex Corporation, Austin, TX) were coupled to the antigens using standard protocols. A total of 30 µg of protein was used for each antigen per 12.5×10^6 bead microsphere coupling reaction. PBS (pH 7.2) was used to couple OV-16-GST and GST control protein, whereas 2-(N-morpholino) ethane-sulfonic acid (pH 5.0) was used for coupling OV-17-GST and OV-33-GST.^{25,28}

Multiplex bead assay. Serum samples were diluted 1:400 in a PBS solution containing 0.5% casein, 0.3% Tween-20, 0.5% polyvinyl alcohol, 0.5% polyvinyl pyrrolidone, 0.2% NaN₃, and incubated overnight at 4°C with crude *E. coli* extract at a final concentration of 3 µg/mL before testing. Coupled beads at 2,500 beads/antigen/well in 50 µL of Buffer A were added to 96-well filtered-bottom plates (Millipore, Bedford, MA) and wash aspirated. Fifty microliters of sample were added in duplicate wells and incubated 1.5 hours at room temperature with gentle shaking.^{20-26,28} All samples were separately screened for IgG and IgG4 responses. Positive controls for each antigen were calibrated to give an expected mean fluorescent intensity (MFI) signal minus the background (bg) noise of between 8,000 and 15,000 MFI-bg. Antibody binding was detected with biotin-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at 1:15,000, or biotin-conjugated mouse anti-human IgG4 (Life Technologies, Carlsbad, CA) at 1:1,250. Secondary antibodies were allowed to react with 1:200 streptavidin-phycoerythrin (Invitrogen, Carlsbad, CA), and 125 µL of washed beads in PBS were read in a Bio-Plex 200 instrument using BioPlex Manager version 6.1 (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis. Threshold values for positive antibody responses, assay characteristics, and 95% confidence intervals (CI) were visualized and analyzed using the pROC package in R version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria).^{37,38} CIs were calculated using bootstrap analyses set at 10,000 stratified replicates. Receiver operating characteristics (ROC) curves were compared using Delong's test for two correlated ROC curves.³⁹

The study was powered to detect differences in accuracy (area under the ROC curve) of at least 0.10 between assays using a two-sided z test. A sample of at least 100 positives and 300 negatives was needed to achieve the required power of > 99.9% with a significance level of 0.05. Descriptive analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Inc., San Diego, CA).

RESULTS

The median MFI-bg among sera from *O. volvulus*-positive individuals and among sera from *O. volvulus*-negative individuals are displayed for each of the antigens for both total IgG and for IgG4 in Table 2 along with the results from the ROC analyses.

Assay performance and determination of cutoffs. OV-16: ROC analysis produced an area under the curve (AUC) of 0.987 for the IgG assay and 0.984 for the IgG4 assays, respectively (Figure 1). The optimal cutoffs for IgG and IgG4 were 586 and 33 MFI-bg, with sensitivities of 95.4%

FIGURE 1. Receiver operating characteristic (ROC) plots for each assay. Optimal thresholds are indicated by the black circles. Error bars represent 95% confidence intervals. This figure appears in color at www.ajtmh.org.

(CI = 92.7–99.1) and 96.3% (CI = 92.7–99.1) and specificities of 99.4% (CI = 96.4–100) and 99.8% (CI = 99.4–100), respectively (Table 2). For the IgG assay, a high cutoff that captured all of the true negative values (100% specificity) reduced sensitivity to 83.5%, whereas a low cutoff affording 100% sensitivity reduced specificity to 3.4%. Maximizing specificity of the IgG4 assay to 100% reduced sensitivity to 95.4%, whereas maximizing sensitivity to 100% reduced specificity to 1.4%.

Using the optimal threshold, the IgG assay misclassified five (4.6%) sera as negatives and three (0.6%) as positives. The three false positives came from one *Wuchereria bancrofti* specimen, one *Entamoeba* spp. specimen, and one U.S. non-traveler specimen, with IgG responses to OV-16 of 777, 795, and 3,451 MFI-bg, respectively. The IgG4 assay misclassified four (3.6%) sera as negatives and one (0.2%) serum as positive. The false positive was a *W. bancrofti* specimen from India, with an IgG4 response to OV-16 of 48 MFI-bg.

OV-17: ROC analysis produced an AUC of 0.912 for the IgG assay and 0.879 for the IgG4 assay (Figure 1). The optimal cutoffs for the IgG and IgG4 assays were 915 and 603 MFI-bg with sensitivities of 86.2% (CI: 70.6–96.3) and 76.1% (CI = 67.0–94.5) and specificities of 79.2% (CI = 69.7–95.8) and 82.8% (CI = 63.9–92.2), respectively (Table 2). For the IgG assay, a high cutoff resulting in 100% specificity reduced sensitivity to 32.1%, whereas a low cutoff affording 100% sensitivity reduced specificity to 36.5%. Maximizing the specificity of the IgG4 assay to 100% reduced sensitivity to 20.2%, whereas maximizing the sensitivity to 99.1% reduced specificity to 0.2%.

The IgG assay misclassified 15 sera as negative and 104 sera as positive (Table 2). The 104 false-positive specimens included 77 with *W. bancrofti* (median = 3,693

FIGURE 2. Antibody responses (MFI-bg, top axis) against three different antigens of *Onchocerca volvulus* by sample types (y axis). Each dot represents an individual serum. Left panels are IgG responses. Right panels are IgG4 responses. This figure appears in color at www.ajtmh.org.

MFI-bg, range (R) = 969–24,193), 11 with *Mansonella ozzardi* (median = 1,994 MFI-bg, R = 938–22,607), six with *Loa loa* (median = 2,529 MFI-bg, R = 1,258–11,067), two with *Toxocara* spp. (1,050 and 1,155 MFI-bg), one with *Schistosoma mansoni* (980 MFI-bg), one with *B. malayi* (2,466 MFI-bg), and six U.S. specimens (median = 2,772 MFI-bg, R = 1,090–20,650).

The IgG4 assay misclassified 26 sera as negatives and 86 sera as positives (Table 2). The false-positive specimens included 74 with *W. bancrofti* (median = 6,150 MFI-bg,

R = 635–28,103), seven with *M. ozzardi* (median = 3,759, R = 857–24,592), two with *L. loa* (2,548 and 9,288 MFI-bg), two Indian endemic controls (2,317 and 21,783 MFI-bg), and one *B. malayi* positive specimen (6,416 MFI-bg) (Figure 2).

OV-33: ROC analysis produced an AUC of 0.984 for the IgG assay and 0.991 for the IgG4 assay (Figure 1). The Ov33 cutoff values for IgG and IgG4 were 5,216 and 67 MFI-bg, with sensitivities of 90.8% (CI = 87.2–99.1) and 96.3% (CI = 93.6–100) and specificities of 96.8% (CI = 87.4–98.2) and 98.6% (CI = 95.8–99.6), respectively. Maximizing the specificity of the IgG assay to 100.0% reduced sensitivity to 33.0%, whereas maximizing sensitivity to 100% reduced specificity to 81.4%. Maximizing the specificity of the IgG4 assay to 100.0% reduced sensitivity to 69.7%, maximizing sensitivity to 100.0% reduced specificity to 33.1%.

Based on optimal thresholds, the IgG assay misclassified 10 sera as negatives and 16 sera as positives (Table 2). The 16 false positives included six *W. bancrofti* specimens (median = 9,473 MFI-bg, R = 5,819–25,031), three *L. loa* specimens (median = 7,650 MFI-bg, R = 5,381–12,508), two *Taenia solium* specimens (10,925 and 24,158 MFI-bg), one *S. mansoni* specimen (10,904 MFI-bg), one *Strongyloides stercoralis* specimen (6,182 MFI-bg), and three U.S. non-travelers (median = 14,276 MFI-bg, R = 8,389–16,533) (Figure 2). The IgG4 assay misclassified four sera as negatives and seven sera as positives (Table 2). The seven false positives included four specimens with *W. bancrofti* (median = 1,371 MFI-bg R = 227–15,428 MFI-bg), two *S. mansoni* specimens (232–1,606 MFI-bg), and one *M. ozzardi* specimen (307 MFI-bg) (Figure 2). One of the false positives with *W. bancrofti* was also positive by the IgG4 anti-OV-16 assay, and was the only sample out of the 501 non-onchocercal specimens that resulted in a false positive in both the IgG4 anti-OV-16 and the IgG4 anti-OV-33 assays. This sample was negative by the IgG4 anti-OV-17 assay.

Of the four sera with false-negative results in the IgG4 anti-OV-16 assay, two were positive in the IgG4 anti-OV-33 assay. Similarly, of the four sera with false-negative results in the OV-33 assay, two were positive in the OV-16 assay. Only two onchocerciasis-positive specimens yielded false-negative results in both assays.

Overall, the coefficient of variation for the positive assay controls was less than 9%, and all negative control sera were consistently below the cutoff values described earlier for all three onchocercal antigens. Beads coupled with GST alone were evaluated in all 610 samples, to determine nonspecific reactivity. Reactivity against GST was limited, with a median MFI-bg of 31 (R = –6 to 1,289) for IgG; the values were lower for IgG4, with median MFI-bg of 1 (R = –6 to 70).

Among all samples, 12 specimens had IgG reactivity to beads coupled with GST alone that was above the threshold for the IgG anti-OV-16 assay. Ten of these 12 samples were negative for OV-16-GST reactivity, and two were positive for OV-16-GST reactivity in excess of 12,000 MFI-bg units. One sample had IgG reactivity to beads coupled with GST alone that was over the positivity threshold for the IgG anti-OV-17 assay (1,289 MFI-bg units), but was negative for OV-17-GST reactivity. No samples had IgG reactivity against GST alone that surpassed the threshold for the IgG anti-OV-33 assay. Two samples had IgG4 reactivity to

GST-only beads that was above the threshold for the IgG4 anti-OV-16 assay (70 and 40 MFI-bg). Both of these specimens were positive for IgG4 anti-OV-16 reactivity in excess 18,000 MFI-bg units (18,812 and 23,238 MFI-bg). No samples had IgG4 reactivity to GST-only beads above the positivity threshold for OV-17 or OV-33.

Comparison of assays. There were no significant differences in the AUC between the IgG and IgG4 assays of OV-16 ($P = 0.62$) or for OV-33 ($P = 0.24$). The AUC for the IgG and IgG4 assays of OV-17 was significantly different ($P = 0.002$). No statistically significant difference was found between the ROC curves for the IgG4 assays of OV-16 and OV-33 ($P = 0.44$) nor between the IgG assays of OV-16 and OV-33 ($P = 0.73$); however, the IgG4 assay of OV-17 performed significantly worse than the IgG4 assays of both OV-16 ($P < 0.001$) and OV-33 ($P < 0.001$).

DISCUSSION

In our analysis, the anti-OV-16 MBA had the highest specificity and sensitivity of those antigens tested, followed closely by the anti-OV-33 MBA. Although no statistically significant difference was detected between the IgG and IgG4 assays for OV-16 and OV-33 (Figure 1), the IgG assays for both resulted in more false positives than the IgG4 assays (Figure 2). This suggests that IgG4 immunoassays are preferable to IgG assays in the elimination context. WHO guidelines for elimination require seroprevalence $< 0.1\%$ to stop MDA.⁷ Therefore, even a few false positives could result in unnecessary continuation of MDA. Furthermore, the IgG4 assays for OV-16 and OV-33 are able to maintain much higher sensitivity when the specificity is maximized. Although it would be ideal to have a highly specific test that was also capable of identifying true infections (e.g., detection of fertile adult females), no test is yet available.

OV-16 IgG4 antibody tests are currently available in other formats, including ELISA and a rapid diagnostic test.^{12,13,40} As such, OV-16 is the antigen most commonly used in serologic test for programmatic testing⁸; however, given the high specificity and sensitivity of the anti-OV-33 IgG4 assay, it has potential for use as a confirmatory test alongside anti-OV-16 in the MBA. The inclusion of two antigens for onchocerciasis in assays for M and E might allow public health practitioners to make rapid determinations in the face of indeterminate or potentially false-positive results. Additionally, M and E programs faced with concerns about missing patent infections, such as when mapping a hypoendemic area, using a combination of tests may reduce the number of missed infections. Furthermore, studies of homologues to OV-33 found in other filarial parasites previously suggested these antigens could be markers of early infection.^{21,22,41–43} If found to be an early marker for onchocerciasis, OV-33 may play a role for the detection of recrudescence in areas that have stopped MDA.

The present study tested only a single batch of coupled beads for each antigen. This was done to avoid variance between batches that could affect cutoff thresholds. Cutoff thresholds are dependent on specific coupling conditions and must be recalibrated for each new batch of antigen-bead coupling that is produced. Although cutoff thresholds are expected to change for each new batch of beads, we

anticipate that overall performance characteristics will remain consistent across couplings.

MBAs, whether using just anti-OV-16 or both anti-OV-16 and anti-OV-33 antigens, could be used for routine surveillance anytime. Blood was collected to evaluate the impact of other public health programs (e.g., NTD, malaria, vaccination) in river blindness program areas, generating information for multiple diseases in a single assay. They would also be helpful when used in combination in areas that are being evaluated to determine if MDA can be stopped, as multiple antigens of *O. volvulus* could be simultaneously tested. The use of the MBA will require specialized training, particularly to ensure proper coupling and determination of cutoff thresholds. It also requires specialized equipment that many programs do not currently possess. However, the needed equipment is becoming more commonly available as various programs take up the technology.

Compared with current surveillance methods for onchocerciasis, the MBA is a more sensitive platform than skin snip microscopy (CDC, unpublished data) and can be a more versatile tool than the IgG4 anti-OV-16 ELISA.¹⁵ The MBA has advantages both as a research tool and as a surveillance tool. Given the many areas of co-endemicity for onchocerciasis and other NTDs, an integrated monitoring platform would be ideal. As elimination programs move toward integration, it makes sense that M and E efforts for different infections should be integrated; the MBA is one tool that would facilitate an integrated surveillance platform. As demonstrated by this study, both OV-16 and OV-33 can discriminate onchocerciasis from other filarial infections and both antigens are good candidates for inclusion in integrated sero-surveillance of NTDs on the MBA platform.

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