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Field test and sensitivity of Onchocerciasis rapid test in Ogun State, Nigeria

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Abstract

Objective: This study, conducted between March 2015 and July 2016, investigated the sensitivity and specificity of the OV-16 RDT and Dried Blood Spot (DBS) Enzyme-Linked Immunosorbent Assay (ELISA) for skin snip microfilaria (MF) detection in Ogun State.

Methods: Five hundred and eighty-eight (588) members of 16 first-line communities in 8 endemic Local Government Areas (LGAs) provided fingerstick whole blood specimens examined for IgG4 antibodies against the *O. volvulus* antigen OV-16, using DBS OV-16 ELISA and OV-16 RDT. In consort with these specimens, 162 study participants consented to be evaluated by skin snip.

Results: Results showed a seroprevalence of 106(18.0%) using ELISA and 102(17.3%) by RDT, with 92.2% agreement. The OV-16 RDT demonstrated a positive and negative agreement of 76.4 (95% CI 67.2 to 84.1%) and 95.6% (95% CI: 93.4 to 97.3), respectively, to the OV-16 ELISA results from the concordant dataset. Against the 64 positive skin snips microscopy results, sensitivity of the RDT was 79.7% (95% CI 73.43% to 85.27) and the ELISA was 70.3% (95% CI 64.72% to 75.37%).

Conclusion: The results of this study provided information on the performance of the OV-16 RDT. This information will contribute data for comparison to studies in other areas where treatment is ongoing. In conclusion, the commercial launch of the OV-16 RDT has made available a point-of-care diagnostic tool that could serve to help local and country onchocerciasis elimination programs to measure changes and progress in onchocerciasis intervention.

Keywords: Onchocerciasis, diagnosis, IgG4 OV-16 RDT, ELISA, Ogun State, Nigeria

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Test sur le terrain et la sensibilité du test rapide de l'onchocercose dans l'État d'Ogun , au Nigéria

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Résumé

Objectif de l'étude: Cette étude, menée entre mars 2015 et juillet 2016, a examiné la sensibilité et la spécificité du test immuno-enzymatique (TIE) du TDR OV-16 et de la tache de sang séché (TSS) pour la détection des microfilaires (MF) dans l'État d'Ogun.

Méthode de l'étude: Cinq cent quatre-vingt-huit (588) membres de 16 communautés de première ligne dans 8 zones de gouvernement local (LGA) endémiques ont fourni des échantillons de sang total prélevés sur le bout du doigt examinés pour les anticorps IgG4 contre l' antigène *O. volvulus* OV-16, à l'aide de DBS OV- 16 ELISA et OV-16 RDT. Parallèlement à ces échantillons, 162 participants à l'étude ont consenti à être évalués par biopsie cutanée.

Résultat de l'étude : Les résultats ont montré une séroprévalence de 106 (18,0 %) par ELISA et de 102 (17,3 %) par RDT, avec une concordance de 92,2 %. Le TDR OV-16 a démontré une concordance positive et négative de 76,4 (IC à 95 % 67,2 à 84,1 %) et 95,6 % (IC à 95 % : 93,4 à 97,3), respectivement, avec les résultats ELISA OV-16 de l'ensemble de données concordant. Par rapport aux 64 résultats positifs de microscopie cutanée, la sensibilité du TDR était de 79,7 % (IC à 95 % 73,43 % à 85,27) et celle de l'ELISA était de 70,3 % (IC à 95 % 64,72 % à 75,37 %).

Conclusion : Les résultats de cette étude ont fourni des informations sur les performances du TDR OV-16. Ces informations fourniront des données pour la comparaison avec des études dans d'autres domaines où le traitement est en cours. En conclusion, le lancement commercial du TDR OV-16 a rendu disponible un outil de diagnostic au point de service qui pourrait servir à aider les programmes locaux et nationaux d'élimination de l'onchocercose à mesurer les changements et les progrès de l'intervention contre l'onchocercose.

Mots-clés : Onchocercose, diagnostic, TDR IgG4 OV-16, ELISA, État d'Ogun , Nigéria

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INTRODUCTION

Onchocerciasis, also known as "river blindness," is an arthropod-borne disease caused by infection with *Onchocerca volvulus*, a parasitic nematode that forms nodules under the skin transmitted by black flies of the genus *Simulium*. It ranks among the highest causes of visual impairment globally [1]. Humans are the natural host, with an estimated 120 million people at risk of infection [2,3]. The disease spans continents, with transmission occurring across rural sub-Saharan African countries, and in a small focus in Yemen in the Arabian Peninsula, and elimination has recently been confirmed in much of the Americas [4,5]. In ivermectin naïve communities of Africa, elimination of onchocerciasis transmission is more difficult because of the presence of *Loa loa* (commonly known as African eye worm) and its associated *Loa*-related severe adverse events SAEs. Also, political instability, insecurity and limited access to endemic areas pose additional challenges (4). Despite these challenges, onchocerciasis has been targeted for elimination in major African countries by 2025 [6].

Over the years, the global impact of the disease on the poor has propelled the World Health Organization and major international health institutions, including pharmaceutical companies, to support programs aimed at controlling the disease. The donation of ivermectin, a safe and effective micro-filaricide for the treatment of onchocerciasis by (Merck & Co., Kenilworth, NJ), resulted in the control of the disease morbidity [7]. Elimination of the disease in Africa now appears possible following a proof of principle that long-term Community-Directed Treatment with Ivermectin (CDTI) targeted at the 15 years lifespan of *O. volvulus* could result in the elimination of the disease [8, 9, 10]. This has shifted the emphasis from control of onchocerciasis to elimination in Africa

In view of this, continuous monitoring of the disease is important to determine the impact of treatment for the advised ending of Mass Drug Administration (MDA) of ivermectin. Diagnosis of the disease had been through the use of skin snip microscopy to detect microfilaria (MF) in the subcutaneous area of the upper iliac crest of an individual [11]. However, the inability to sufficiently detect early infections and low MF density by skin snip microscopy is one of the numerous challenges encountered in the quantification of onchocerciasis prevalence in endemic areas [12]. Also, MDA surveillance in low-resource settings lacking sophisticated instrumentation has been a challenge to the global efforts to the elimination of onchocerciasis. The Immunoassay of the *O. volvulus* filarial antigen (OV-16) has been found to be highly specific [13-17]. The OV-16 immunoassay as an alternative to the invasive skin snip method has the potential for mapping and post-MDA surveillance in Africa where highly prevalent coincident filarial infections (*Loa loa* and *Mansonella* spp.) confound less specific immunoassays [18]. Most useful, however, for programmatic needs would be rapid diagnostic tests (RDTs) where central laboratory processing is not needed. Recently, a commercial non-invasive rapid assay test kit that is capable of detecting IgG4 antibodies specific to OV-16 antigen—intended for post-treatment surveillance and point-of-care assessment from fingerstick blood specimens—has become available [19]. The correct application of this RDT may provide a field-deployable alternative to OV-16 ELISA in identifying communities at greater risk of infection and transmission of onchocerciasis, including evidence of previous infection in cases with no MF count. Communities will also receive priority attention to prevent further progression of morbidity that may result in blindness and thus, impact on the person's productivity and quality of life [20].

The launch of a commercially available OV-16 rapid assay portends to be a game changer in the advancement towards onchocerciasis elimination. Nonetheless, information on the field performance of commercial OV-16 RDT onchocerciasis elimination is important to guide policy direction. This study therefore investigated the performance of the OV-16 RDT in comparison with DBS ELISA and skin snip microscopy for essential programmatic and implementation resolutions.

MATERIAL AND METHODS

Study Area

The study took place in sixteen first-line CDTI communities in eight Local Government Areas (LGAs) in Ogun State.

Sample size determination

Sample size was determined using the formula as described by Pourhoseingholi *et al* (2013).

$$n = 1.96^2 p(1-p)/d^2$$

The prevalence of onchocerciasis for each LGA was estimated as 38.9% based on the average value of a wide range of publications in this area (10). The minimum sample size for the study was determined to be approximately 300.

Inclusion criteria

Communities that have been receiving treatment via MDA with ivermectin for over 10 years were selected for the study based on their consistency of treatment. Treatment information was verified from treatment records obtained from the Ogun State Ministry of Health.

Ethical approval

The study was approved by the ethics review committee of the Ogun State Hospital Management Board, Abeokuta and the Ministry of Health (SHA/RES/VOL2/153). Informed Consent Forms are duly signed by voluntary participants and by parents or guardians of child participants. This study was conducted in accordance with the declaration of Helsinki and confidentiality was preserved for all data collected.

Serological Evaluation using OV-16 RDT

A total of 588 study participants provided fingerstick whole blood for the OV-16 RDT, blood was also stored on Whatman™ 903 Protein Saver Cards (GE Healthcare, Pittsburgh, PA) to produce dried blood spots (DBS). Of these, 588 participants' fingerstick whole blood specimens assessed by both OV-16 RDT and OV-16 ELISA, a further subset of these participants (n=162) consented to be skin snipped for viewing under the microscope.

Collection of Dried Blood Spots on Whatman® Protein Savers Card for OV-16 ELISA

Each Whatman paper card was labeled with a participant ID. Fingerstick whole blood on the paper was dried under ambient conditions during the day then stored at 10 cards per re-sealable mylar pouch containing two units clay desiccant packets (Desiccare, Reno, NV) and a humidity indicator card to confirm humidity <20% in the pouch before laboratory analysis. DBS were stored in the field in coolers containing icepacks and were transported to the university laboratory within 2 to 5 days of collection and stored at 4°C until shipment. Dried blood spots were expressly shipped at ambient temperature to the PATH laboratory (Seattle, Washington, United States) for ELISA testing. Once received at PATH, DBS are stored at -20°C in sealed desiccant-containing pouches until use.

Enzyme Linked Immunosorbent Assay for Dried Blood Spots

An OV-16 ELISA protocol using a horseradish peroxidase detection conjugate was used as previously described [19-22]. Immulon 2HB (Thermo Fisher Scientific, MA, USA) plate wells were coated with 100µL of 5µg/mL OV-16 antigen diluted in phosphate-buffered saline (PBS), pH 7.4 (Sigma MO, USA) overnight at 4°C. DBS were punched with a 6 mm circular punch and each punched DBS was eluted overnight in 200µL of phosphate-buffered saline with 0.05% Tween-20 (PBST) with 2% (w/v) non-fat dry milk (Sigma). Control DBS were prepared from blood samples containing the anti-OV-16 positive control antibody: a solution of 250 ng/mlanti-OV-16 recombinant IgG4 clone AbD19432_hIgG4 (Bio-Rad AbD Serotec, Puchheim, Germany) in pooled normal human plasma (Basematrix 53, Seracare, MA, USA) was mixed thoroughly at a 1:1 dilution with packed, washed, O-negative normal donor red blood cells (Plasma Lab International, WA, USA). Control blood was applied to Whatman 903 paper at 15 uL per 6 mm pre-punched circle, dried overnight in ambient laboratory conditions, then stored with desiccant at -20°C until use.

Each plate included one punch of the positive control DBS, eluted in the same manner as a test DBS punch. Following overnight OV-16 antigen coating, plates were blocked with phosphate-buffered saline with 0.05% Tween-20, pH 7.4 (PBST, Sigma, MO, USA), + 5% fetal bovine serum (FBS, Thermo Fisher, MA, USA) at 37°C. Plates were then washed three times with 300 µL per well with PBST and tapped dry. DBS eluate samples were added without dilution to the plate at 50µl per well. An anti-OV-16 IgG plate positive control was previously prepared by diluting the OV-16 positive control IgG4 antibody (part number AbD19432.1, Bio-Rad, Raleigh, NC, USA) into PBST and 5% Basematrix 53, to a final concentration of 2.5 ng/mL antibody, and stored at -80°C until use. For each ELISA, this plate positive control was thawed and added without further dilution, 50 µL each well to 4 wells per ELISA plate.

Samples and controls were incubated at 37°C for one hour and then washed three times with PBST. A 1:5,000 dilution of an anti-human IgG4 (6025 clone Hybridoma Reagent Labs, Baltimore, MD) was added at 50µl per well. Plates were incubated at 37°C for one hour and then washed four times with PBST. A 1:10,000 dilution of a horseradish peroxidase (HRP)-conjugated goat, anti-mouse antibody (115-035-062-HRP, Jackson Immuno Research Labs, PA, USA) was added at 50µl per well. Plates were incubated at 37°C for one hour and washed four times with PBST. 100µL of TMB (Sigma) solution was added to each well. Plates were

incubated at room temperature for 15 minutes and then the reaction was stopped by adding 50µl per well of 1N HCl (Thermo Fisher).Plates were read at 450nm. Replicate well ELISA units were averaged.

Parasitological Examination

Microscopic examination of MF under superficial skin snips was conducted according to previously described methods [10, 23].

Data analysis

ELISA optical density (OD) measurements were subjected to quality control criteria prior to analysis. Replicate sample and control well results were tested against criteria for difference in OD measurements to ensure that individual well measurements did not exceed statistically expected variation for replicates (>26% CV for replicate sample wells, >20% for plate positive control wells). If passed, the sample and control OD measurements were averaged to produce the mean sample OD and included in the ELISA analysis. If criteria were not met, the samples were repeated when additional specimen was available. The mean OD measurements for samples were normalized relative to their respective plate DBS positive control mean OD measurement. Two methods were used to determine a threshold for ELISA positivity. First, a univariate expectation-maximization (EM) algorithm was applied to the normalized OV-16 ELISA dataset OD measurements to identify two sub populations in the data as OV-16 positive or negative. The EM algorithm was set to model the normalized distribution of OD measurements with two Gaussian curves, resulting in a threshold for positivity of 0.34 normalized OD. In the second method, a negative peak within the distribution of the normalized OD values was identified by calculation of the median of the entire dataset. Two times the median (2X median) was used to define the threshold for positivity shown in Figure 2. The 2X median method and resulting threshold of 0.36 normalized OD was used for classification of the final dataset and performance comparisons. Sensitivity of ELISA and OV-16 RDT were calculated against positive skin snip microscopy as the percentage of positive by either assay out of all MF+ results (n=64). OV-16 RDT was also compared to OV-16 ELISA and both degree of positive and negative agreements were calculated against all ELISA results (n=588) as proportions of RDT result versus reference ELISA result.

RESULTS

Study adherence

Of the 588 participants that enrolled and took part in the OV-16 RDT and DBS ELISA study,162 of them agreed to participate in the skin snip for MF microscopy (Figure 1).

Positive and negative agreement of OV-16 RDT to OV-16 ELISA

From the 588 concordant participant ID OV-16 RDT and OV-16 ELISA data set, ELISA seropositivity of each DBS for IgG4 antibodies against OV-16 antigen was determined through comparison of the normalized value (mean sample OD/mean plate positive control OD) to the threshold value of 0.36 (Figure 2). Binary OV-16 RDT results were classified relative to reference OV-16 ELISA positive and negative values as the following: true positive (TP), false positive (FP), true negative (TN), and false negative (FN) (Table 2). Degree of the positive and negative agreement of the RDT performance as compared to ELISA were calculated to be 76.4% (95% CI 67.2 to 84.1)and 95.6% (95% CI: 93.4 to 97.3), respectively.

Sensitivity of OV-16 RDT and ELISA results to reference skin snip microscopy.

Of the 162 skin snip microscopy results from participants for which there were both RDT and ELISA results, 64 were positive for microfilaria (MF+). Sensitivities of RDT and ELISA to MF+ were calculated to be 79.7% and 73.4%, respectively. Comparative performances of the OV-16 RDT between skin snip microscopy (MF), RDT, and ELISA are presented in Table 1. A seropositive rate of 17.3% (102/588) by RDT and 18.0% (106/588) by ELISA with 92.2%agreement was recorded. Across different age groups, similar percent positives were observed for both the ELISA and the RDT (Figure 3). There was no significant variation between the percent prevalence as determined by OV-16 RDT and ELISA with respect to different age categories ($p > 0.05$) using Chi square.

Characterization of Assay-concordant and Assay-discordant results.

Performance categories of the RDT and ELISA as compared to reference assay (RDT to both ELISA and skin snip microscopy, ELISA compared to skin snip microscopy) are plotted against the normalized ELISA values. False-negative results for RDT versus ELISA had lower median of normalized ELISA value (Figure 4) as

compared to RDT results, which were true-positive in agreement with ELISA. In contrast, false positive results for RDT versus ELISA had a higher median normalized ELISA value as compared to RDT true-negative results in agreement with ELISA. This suggests that both types of discordant results trend towards the cutoff for the ELISA and may represent a level of specific signal prone to high uncertainty in both assay types.

DISCUSSION

Human IgG4 antibody reaction to OV-16 antigen has been recognized as a serologic marker of exposure to *O. volvulus* in monitoring of progress toward elimination of river blindness [15, 24]. In the Onchocerciasis Elimination Program of the Americas and some selected foci in Africa, the OV-16 ELISA had been used as a tool to confirm transmission interruption and elimination [25, 26]. Reports on performance of previous versions of an OV-16 rapid test by previous researchers [27, 28, 16] suggest the practical utility of an OV-16 RDT for serological assay of exposure to the OV-16 antigen. To this end, an accurate and commercially-available OV-16 RDT could be an important tool to measure progress in MDA with ivermectin.

This study reports the performance of the current OV-16 RDT as used in a field setting in an area undergoing MDA with reference assays of both OV-16 ELISA and skin snip microscopy. Additional data of this type of diagnostic performance testing is critically needed to understand the full performance and utility of such the RDT in different populations and prevalence settings. It has been acknowledged that some fraction of individuals exposed to the *O .volvulus* parasite may not make a detectable IgG4 response to OV-16 antigen [29]. This phenomenon is also seen within this data set; while a greater proportion of MF+ specimens had higher normalized ELISA values, there are also low normalized ELISA values. This also supports that some proportion of MF-positive individuals will not be detected by OV-16 serology (Figure 2B). A challenge in analysis of the performance of the RDT has been that the performance of the reference OV-16 ELISA across Africa has not been operationally well-understood. It is thought that OV-16 ELISA sensitivity may be under 50%, though exhibiting a high degree of specificity [30]. Performance of any serological assay may be highly dependent on the population tested, method, and sample types used to define the threshold. Because of the possibility of variable serological baselines, previous studies have employed baseline-diagnostic approaches such as expectation-maximization (EM) to identify the two populations [31, 32]. A possible limitation of EM is that it seeks to define two peaks. The OD distribution of putative positives is quite broad and may have a poorly defined peak.

In this study, the 2X median method was employed to fully capture the location of the negative peak. This method assumed the negative peak to be normally distributed such that twice the median would capture the majority of the negatives in this dataset and potentially could be used with other sets where there is only a clearly identifiable negative peak. The 2X median method was chosen methodologically as a simple method that leverages the increasingly high proportions of negative results in datasets, such as from serological surveys where control and treatment have been implemented. There are limitations to this approach to define a threshold. The median could be heavily impacted in a scenario where there were many positives, in which case EM could be a better choice. As described in methods, EM was also used on this study set. The threshold was identified by EM to be 0.34, as opposed to 0.36 identified using the 2X median method. When EM was used to analyze the identical normalized dataset, the resulting threshold was found to be 0.34. The slight shift in threshold when using EM resulted in a sensitivity of 72.6% and 95.8% specificity, as compared to 76.4% and 95.6% found when using the 2X median method. There was no impact of using the EM-derived threshold on the sensitivity of ELISA to skin snip-positive samples; it was found to be the same as when ELISA positivity was determined by the 2X median method, 73.4%.

In this study, the ELISA was normalized to a single positive control point. Comparison to standard curves with the threshold determined based on prior study sets has been used in other studies [33, 34, 35] however they are used such that they do not produce a fundamentally different result than single point normalization used in conjunction with plate quality controls. The standard curve serves as a plate quality control element, but a single concentration serves to determine positivity, which may provide little differentiation from the strategy of single point normalization compared to a threshold, with other plate quality control elements, as was done in this study. Furthermore, the use of a standard curve creates a higher risk of user error and variability in the data than using premade

controls of same-type matrix (DBS) at specific concentrations. Calculation of concentration using the standard curve of monoclonal antibody positive control may provide a more accurate value relative to the standard curve, but as anti-OV-16 IgG4 from individuals is expected to be highly polyclonal, it is only an approximate concentration and carries the same risk of error introduced with standard curve preparation. In future studies, better refinement of an appropriate threshold will be possible given the goal of wide implementation of a harmonized OV-16 ELISA method [35].

The degree of positive and negative agreement of the OV-16 RDT when compared to the OV-16 ELISA as analyzed by the 2X median method was 76.4% and 95.6%. Both an imperfect outcomes were seen for the RDT against the ELISA despite being assays for the same target analyte. Discordant results (false positive and false negative) of RDT to ELISA show distributions closer to the threshold of the ELISA normalized value. Furthermore, of those RDT results classified as false positive against ELISA or false negative against ELISA for which there was a skin snip result (n=10/21 and n=12/25, respectively), 8/10 of RDT false positive to ELISA and 4/12 of RDT false negative to ELISA were MF+. This suggests that those results discordant by RDT against ELISA are reflective of limitations of both test and reference assays at low OV-16-specific IgG4 levels. This also explains the slightly higher observed sensitivity of the RDT to MF+ than ELISA and imperfect specificity.

The degree of agreement between OV-16 RDT, ELISA, and skin snip microscopy is important to understanding the performance of the RDT. While skin snip microscopy could confirm whether individuals had a current infection, it was impossible in this study to confirm sero-status by any other method than ELISA. To that end, the RDT performed with relatively high specificity against the ELISA, and, as noted, those discordant results were likely reflective of the integrated limits of both the RDT and the ELISA, particularly given the higher number of RDT false positives that were indeed skin MF+.

The calculated sensitivity of OV-16 RDT to skin snip or ELISA degree of agreement in this study was not as high as previously obtained, although may in similar or higher than those observed in operational analysis of OV-16 ELISA [20, 27, 29]. Limitations of both RDT and ELISA assays may contribute to these discordant results, but further data from study sites of relevance are needed to confirm if different populations, endemicities, and MDA may contribute to changes in observed performance of assays.

The ability of the OV-16 RDT to estimate seroprevalence in areas with ongoing treatment is very important considering the observed limitation of MF detection in low-density infections and in pre-patent individuals. Importantly, skin snip microscopy is becoming unacceptable from a community standpoint. In this study, community members majorly refused to be skin snipped. This singular factor could be seen in the value of skin snip recorded. Their refusal was attributed to the painful nature of the method. However, community members were motivated to wait for up to 20 minutes to know their result using the OV-16 RDT, highlighting that, unlike ELISA, the OV-16 RDT can enable community communication about onchocerciasis treatment, encourage testing, and possibly provide incentive to participate in needed MDA. The test yielded valid results 100% of the time; no invalid results were observed during the study.

Despite the duration of MDA in Ogun State, the continuous presence of MF in communities (though with low densities) could be attributed to the presumed inconsistent use of ivermectin due to pregnancy, fluidity of human movement, and absenteeism [20].

The results of this study provided much-needed information on the performance of the OV-16 RDT, as there is a paucity of information on the field use of the kit. Information from this study will contribute data for comparison to studies in other areas where treatment is ongoing. In conclusion, the OV-16 RDT is a point-of-care diagnostic tool that could serve to help local and country onchocerciasis elimination program to measure the changes and progress in onchocerciasis interventions using the MDA.

Limitations of the study: We consider the following as major limitation in the study. Firstly, our inability to conduct skin snips on all study participants which was due to limited quantity of the scleral punches and this hindered our process as it took time to sterilize the materials. Secondly, the general perception of the painful process of the skin snips device, a handful of the study participants opted for the finger prick blood collection for the OV-16 RDT.

Authors Contributions: GA, YL and DST developed all assays described in the article,

SOA, SSO, FD, AK, AMA and BSO designed and ensured implementation of the study design and data collection activities. SOA, GA, YL, KM and SE performed the data analysis and preparation of the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that they have no competing interests.

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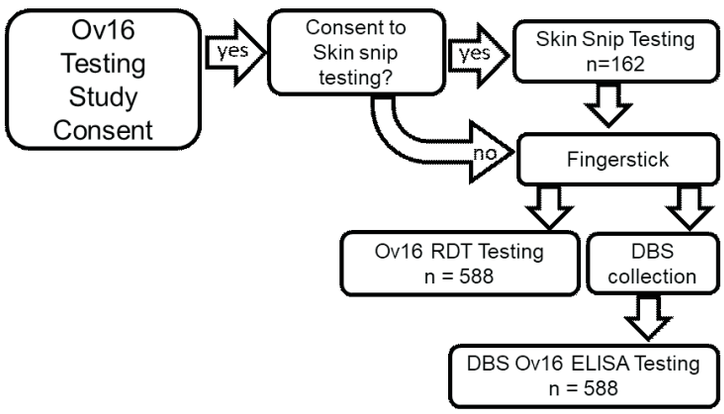

Figure 1. Flowchart of study adherence

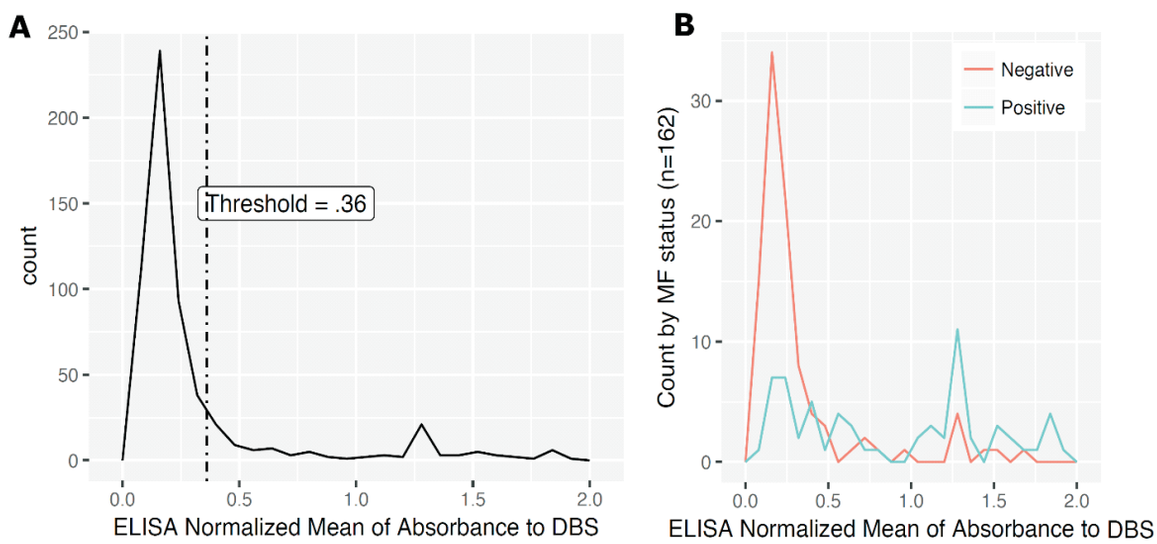

Figure 2. A. Histogram of normalized absorbance (mean sample OD/mean positive control OD) for OV-16 enzyme-linked immunosorbent assay (ELISA) data for participants with DBS OV-16 ELISA results (n=588). B. Histogram of normalized absorbance ELISA data for participants with skin-snip microscopy results (n=162), color-coded by MF-positive (blue line) or MF-negative (red line) results.

Table 1. Summary of Comparative concordance between test RDT, ELISA and skin snip MF results (n=162)

	Skin snip MF positive (MF+, n=64)	**Skin snip MF negative (MF-, n=98)**	**Total**
OV-16 RDT positive	51	13	64
OV-16 RDT negative	13	85	98
OV-16 ELISA positive	47	19	66
OV-16 ELISA negative	17	79	96
Sensitivity RDT to MF+, %	RDT to MF+: 79.7 (95% CI: 73.43 to 85.27)		
Sensitivity, ELISA to MF+, %	ELISA to MF+: 73.4 (95% CI: 67.21 to 79.48)		
Agreement RDT: ELISA	86.4%		

Table 2. Summary of Comparative concordance between test RDT, ELISA for all study samples (n=588)

	OV-16 ELISA positive	OV-16 ELISA negative	Total
OV-16 RDT positive	**81**	**21**	**102**
OV-16 RDT negative	**25**	**461**	**486**
RDT to ELISA positive agreement in %	**76.4** (95% CI 67.2 to 84.1)		
RDT to ELISA negative agreement in %	**95.6** (95% CI: 93.4 to 97.3)		
Overall Agreement RDT:ELISA	92.2%		

Age Category	5-9	10-14	15-19	20-29	30-49	50 +	Overall
Ov16 ELISA-positive	16.7%	17.5%	20.0%	21.2%	20.6%	14.1%	18.0%
Ov16 RDT-positive	12.5%	20.0%	10.0%	21.2%	19.7%	14.7%	17.3%

Figure 3: Seropositive by concordant OV-16 RDT and OV-16 ELISA. Number of RDT-positive and ELISA-positive results shown as compared to total analyzed per each age group. Percent seropositive of each age category is calculated as percent positive/total tested per age group.

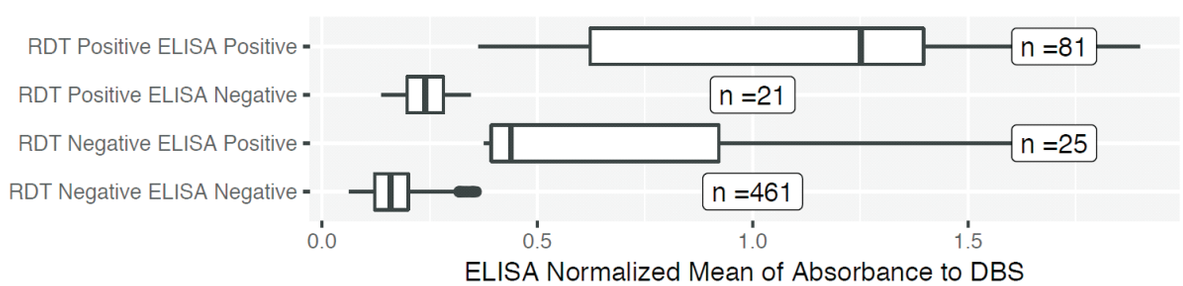

Figure 4. Performance characteristics of the OV-16 RDT vs. ELISA. Median normalized ELISA values for performance categories of RDT compared to ELISA: True positive (RDT and ELISA positive), False positive (RDT negative, ELISA positive), False negative (RDT positive, ELISA negative), and True negative (RDT and ELISA negative). Boxes represent the interquartile range of the ELISA values for each category, with the median marked by the bold line within the box and total numbers in each category are listed to the right of each box.