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Expression and Evaluation of Wb-SXP-1 and Wb-123 Recombinant Antigens as Potential Diagnostic Biomarkers for Lymphatic Filariasis

Sharlene Kerubo Mageto[1*], Rebecca Wanjiku Waihenya[2], Anne Wanjiru Mwangi[3,4], Peter Kipkemboi Rotich[5], Matthew Mutinda Munyao[5], Tonny Teya[5], Robinson Mugasiali Irekwa[5], Joanne Jepkemei Yego[3,5], Caroline Wangui Njoroge[5], Grace Ng'endo Kanyita[6], Nicole Sian Tanchu[3], Dawala Koromtili Oumar[1], Primrose Muthoni Ndungu[7], Samson Muuo Nzou[3,5]

[1]Department of Molecular Biology and Biotechnology, Pan-African University Institute of Basic Sciences, Technology and Innovation, Nairobi, Kenya
[2]Zoology Department, Jomo Kenyatta University for Agriculture and Technology, Nairobi, Kenya
[3]Nagasaki University Institute of Tropical Medicine, Kenya Medical Research Institute (NUITM-KEMRI), Nairobi, Kenya
[4]Production Department, Kenya Medical Research Institute, Nairobi, Kenya
[5]Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya
[6]Department of Biochemistry, Microbiology, and Biotechnology, Kenyatta University, Nairobi, Kenya
[7]Department of Pharmacology, Clinical Pharmacy and Pharmacy Practice, University of Nairobi, Nairobi, Kenya
Email: *sharlenemageto@gmail.com, rwaihenya@jkuat.ac.ke, mwangianne7@gmail.com, pkemboi.peter@gmail.com, emutinda2010@gmail.com, tonyteya15@gmail.com, robinsonmugo@gmail.com, joanneyego@gmail.com, cnjoroge851@gmail.com, graciengendo@gmail.com, sianicoller@live.com, oumarkormtili@gmail.com, primrosendungu@gmail.com, nzoumuuo@gmail.com

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Abstract

Lymphatic filariasis (LF) remains a public health concern as it can cause permanent morbidity and disability to those infected. While the global elimination of LF in these endemic areas is ongoing through mass drug administration, there is the need to develop diagnostic tools that would be utilized to track the progress of total global eradication as well as perform surveillance for the recurrence of lymphatic filariasis transmission. Currently, approved LF diagnosis tools are faced with lack of specificity, low sensitivity, and periodicity dependence. Recombinant filarial antigen-based assays can address these drawbacks and offer practical instruments for LF diagnosis and surveillance. This present study, evaluated rWb-SXP-1 and rWb-123 antigens as potential diagnostic biomarker tools for *Wuchereria banchrofti* in human sera using microspheres-based multiplex serological assay. Based on statistical analysis using XLSTAT 2019 (Addinsoft) on data generated from multiplex technology assay, generated ROC curves for both rWb-SXP-1 and rWb-123

demonstrated 87.1% sensitivity to *Wuchereria banchrofti* human sera with rWb-SXP-1 antigens having the highest specificity of 96%. Indication that rWb-SXP-1 and rWb-123 antigens are capable of detecting immunoglobulin G4 (IgG4) antibodies in human sera synthesized specifically against *W. banchrofti* infections. Therefore, rWb-SXP-1 and rWb-123 antigens can be utilized to detect *W. banchrofti* infections by antibody profiling with excellent diagnostic sensitivity and specificity using microsphere-based multiplex serological tests. This method can be particularly practical for screening a large number of sera samples and/or for quick, extensive field-testing due to the high-throughput and quick formats applied.

Keywords

Lymphatic Filariasis, Recombinant Antigens, Diagnosis

1. Introduction

Lymphatic filariasis (LF) is a tropical disease commonly occurring in low-income populations. Ninety percent of LF is caused by helminths *W. banchrofti*, while-*Brugia malayi*, and *Brugia tumor* parasites are responsible for the remaining ten percent [1] [2] [3]. Female mosquitoes of *Anopheles*, *Culex*, *Aedes*, and *Mansonia* species are the main vectors of microfilariae transmission [3]. LF results in permanent physical impairment when treatment is not done early enough, causing socioeconomic hardship for individuals affected [4] [5] [6] [7].

Diagnosis procedures play vital roles in the monitoring and evaluation of elimination and eradication of LF infections in endemic regions. Transmission assessment surveys TAS programs are dependent on the detection of circulating filarial antigens in human sera utilizing Filariasis Test Strip (FTS), Brugia Rapid test and examination of thick smears (20 - 60 μL) of finger-prick blood using Microscopy technology tests recommended by WHO [8]. These tests are challenged with circulating filarial antigens often remaining detectable in human blood for years after treatment has cleared microfilaria.

Microscopic examination of thick smears from finger-prick blood has been identified as not being a completely reliable and feasible method due to the possibility of inadequate sampling, inconveniences caused by late-night sample collection times, and limited sensitivity, particularly in people with low microfilaria density [9] [10] [11]. Additionally, the ability to identify low levels of infection or recurrence of transmission is limited by the production of microfilaria and antigens months or even years following exposure to LF parasites.

The introduction of CFA detecting tests, Og4C3 ELISA and Immuno-chromatographic card test (ICT) tests was thought to have brought some limelight on the detection and control of filariasis program due to their advantages. However, studies have shown that ICT losses sensitivity in low endemic settings, especially in people exhibiting low levels of circulating filarial antigen and cross-reactivity

with antigens from the related filarial species especially *Loa loa* in Africa remain to be a great challenge towards this main goal of LF elimination [12] [13] [14]. Therefore, these fundamental limitations encountered with the current diagnostic techniques, is likely to support resurgence of Lymphatic filariasis infection in previously declared free endemic area thus dragging behind the goal of total elimination and eradication of LF worldwide.

In order to prioritize where and what interventions to be considered concerning lymphatic filariasis infections, there is a renewed interest in developing specific, sensitive, cost-effective methods for the evaluation of the parasitological status of populations [15]. The preponderance of IgG antibodies to target antigens is important for long-term monitoring and surveillance of infections such as NTDs in populations [16]. Characterization of transmission dynamics in endemic regions by identifying the presence of IgG antibodies specific to *W. banchrofti* parasites in the immune systems of individuals will provide vital information about exposure to these pathogens [17]. Thus, LF diagnostic tools based on antibody tests would be critical for ensuring the success of LF elimination and eradication, since they would form the basis for an alarm system that would track any future contact with *W. banchrofti* parasite.

Recombinant Wb-SXP-1 and Wb-123 antigens have been identified in all stages of *W. banchrofti* life cycle [18] [19]. Therefore the present study evaluated the usefulness of Wb-SXP-1 and Wb-123 recombinant antigens in the identification of immunoglobulin G4 (IgG4) antibodies in human sera synthesized against filarial antigens as biomarkers for diagnosis of lymphatic filariasis using multiplex technology. This was with the intention of developing microsphere based multiplex serological assay that is reliable, quick, cost-effective, specific, and sensitive applicable in detection of *W. banchrofti* infection with minimal hardships.

2. Material and Methods

2.1. Ethical Statement

The Jomo Kenyatta University of Agriculture and Technology Internal Ethical Review Committee approved this study under the reference number JKU/IERC/02316/0586.

2.2. Wb-SXP-1 and Wb-123 Recombinant DNA Cloning

The Wb-SXP-1 and Wb-123 recombinant DNA were already cloned in the pET52b expression vectors separately and stored at the –80˚C freezer at the Kenya Medical Research Institute (KEMRI), Nagasaki University Institute of Tropical Medicine (NUITM) Laboratory.

2.3. Wb-SXP-1 and Wb-123 Recombinant Protein Expression

The Wb-SXP-1 and Wb-123 recombinant DNA were transformed into ECOS-BL21 (DE3) (Code#312-065334) chemically competent cells. 10 µL of ECOS-BL21 (DE3) competent cells were dispensed into two separate 2 mL Eppendorf tubes labeled

Wb-SXP-1 and Wb-123 recombinant DNA respectively. Then 1 µL of pET52b expression vectors carrying Wb-SXP-1 and Wb-123 recombinant DNA respectively was added into each tube, mixed by tapping and left to disperse evenly for 5 minutes on ice [18]. The cells were heat shocked for 45 seconds to create pores in the bacteria cells membranes thus allowing entry of plasmids carrying Wb-SXP-1 and Wb-123 genes into the competent cells. Thereafter the cells were placed on ice for 2 minutes and were separately cultured in 10 mL LB broth with 10 µL carbenicillin then incubated overnight at 37˚C while shaking at 230 rpm in the incubator shaker (INNOVA 42).

After the overnight night incubation at 37˚C, turbidity on the LB broth medium was assessed by looking at cloudiness of the culture medium to confirm the growth of the cultured cells. Thereafter the cultures were separately transferred into large cultures of 500 mL LB broth with 500 µL carbenicillin then incubated at 37˚C while shaking at 230 rpm for approximately 1 h and 45 minutes. Then the cells growth density was measured using the DEN-1B McFarland densitometer (Waken Teck. Co., Ltd.) with the aim of attaining a range of 2.5 - 3.0 density.

After attaining the cell growth density of 3.75 cell/mL and 3.91 cells/mL for the Wb-SXP-1 and Wb-123 genes respective cells, protein expression was induced by the addition of 500 µL of 1 M IPTG (Isoproplyl-β-d-1-thiogalactopyranoside) (Lot# CTJ1296, Thermoscientific, Inc.) to the final concentration of 3.75 cells/mL for Wb-SXP-1 and 3.91 cells/mL for Wb-123 cultures. This was incubated at 37˚C while shaking at 230 rpm for 1 hour. Thereafter density was measured to assess cells growth using the DEN-1B McFarland densitometer (Waken Teck. Co., Ltd.).

The ECOS-BL21 (DE3) (Code#312-065334) chemically competent cells Cultured cells were harvested by centrifugation of the cultures at 5000 xg 20˚C for 15 minutes, and the pellet was obtained. To extract expressed Wb-SXP-1 and Wb-123 recombinant proteins the respective pellets were suspended by pipetting into 10 mL Bugbuster (Cat#70921-50ML, Novagen) containing 1 µL (250 unit) Benzonase Nuclease (Lot#SLBR3250V, Novagen) essential for degrading liberated nucleic acids and 1 µL (30 Kunits) rLysozyme (Catalog Number #7110-1200KU, Novagen) essential for cells lysis. Thereafter the suspensions were transferred into 50 mL Eppendorf tubes and rotated slowly at room temperature for 15 minutes to solubilize the proteins then centrifugation was done at 12 Krpm, 4˚C for 10 minutes and the supernatant was obtained as crude protein extracts. Wb-SXP-1 recombinant proteins extract was obtained as soluble fraction while the Wb-123 recombinant protein extract was insoluble fraction that contained inclusion bodies.

2.3.1. Wb-123 Recombinant Proteins Inclusion Bodies Preparation

Wb-123 recombinant proteins extract pellet was suspended in 10 mL 1x Bugbuster by hard pipetting, followed by addition of 1 µL rLysozyme and the mixture was rotated slowly at room temperature for 15 minutes. Thereafter 10 mL of

1/10 Bugbuster was added, mixed by agitation for 1 minute. The suspension was centrifuged at 5000 xg for 15 minutes at 4˚C and the obtained pellet was suspended into 20 mL of 1/10 Bugbuster in order to wash it and vortexed for 1 minute. Again, the suspension was centrifuged at 5000 xg for 15 minutes at 4˚C and the washing step was repeated twice. After the final washing step, obtained pellet was suspended in 10 mL of 1/10 Bugbuster by agitation for 1 minute.

2.3.2. Wb-123 Recombinant Proteins Inclusion Bodies Solubilization

Wb-123 recombinant protein suspension obtained from the inclusion bodies preparation was centrifuged at 12 Krpm, for 15 minutes at 4˚C. To the inclusion bodies pellet obtained, 10 mL of solubilization buffer (0.3% N-LS/CAPS, PH 11) was added and suspended by hard pipetting for 1 minute. This was followed by rotation at room temperature for 15 minutes and spinning down at 12 Krpm for 15 minutes at 4˚C and the supernatant was obtained as soluble Wb-123 recombinant proteins extract ready for use in the subsequent steps. Dialysis was done to increase the concentration of rWb-SXP-1 and rWb-123 protein respectively. Wb-123 recombinant antigens were dialyzed in 1 litre solution of 0.1% N-LS/PBS (−), PH 7.6 at 4˚C while Wb-SXP-1 recombinant antigens were dialyzed in 1litre solution of PBS (−), PH 7.6 at 4˚C for a period of 2 hours. Dialysis medium was removed after 2 hours, replaced with fresh dialysis medium, and then dialyzed again for 2 hours. After the 2 hours of dialysis, medium was replaced with fresh dialysis medium and then left to dialyze overnight at 4˚C.

2.4. Wb-SXP-1 and Wb-123 Recombinant Proteins Purification

Immobilized Metal Affinity Chromatography (IMAC) using TALON-Accept resin (Code #635503, Takara Bio Inc.) was used to purifyr Wb-SXP-1 and rWb-123 expressed proteins according to the manufacturer's instructions with slight modification [20]. Then His-accept resin was transferred into an empty column (Bio-Rad), washed with double distilled water and then equilibrated using 4 mL of dialysis buffer.

10 mL of rWb-SXP-1 and rWb-123 proteins soluble fractions supernatants were loaded to the His Accept resin and the His flow through (His-FT) solution was collected. Thereafter the His Accept resin was washed 4 mL of 1x Bugbuster and then washed twice with 10 mL NPT-I10 solution. Then respective rWb-SXP-1 and rWb-123 proteins were then eluted using NPT-I1300 elusion buffer and the His elute solution was collected.

2.5. Wb-SXP-1 and Wb-123 Recombinant Proteins Quantification

The Pierce BCA protein assay kit (Thermo Scientific, USA) [20] was used to determine the quantity of pure rWb-SXP-1 and rWb-123 expressed proteins [21]. The standards solutions and working reagents were prepared. 25 µL of each standard solutions, rWb-SXP-1 and rWb-123 proteins samples were separately transferred into tubes labeled respectively. Then 500 µL of working reagent was added into each tube, covered with aluminum foil, and then incubated on water

bath at 37˚C for 30 minutes. Wb-SXP-1 and Wb-123 recombinant proteins were quantified using the BCA protein assay (562 nm) on Thermoscientific Nano-drop 2000c UV-Vis spectrophotometer machine (USA). Absorbance for negative control was used as background and measurements were taken from low to high concentration of the standard solutions, then for Wb-SXP-1 and Wb-123 recombinant proteins all within 10 minutes.

2.6. Wb-SXP-1 and Wb-123 Recombinant Proteins Quality and Purity

Two separate gels were prepared where one was used to assess quality while the other to assess purity of Wb-SXP-1 and Wb-123 proteins. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the purified proteins on the two 10% gels (Irumva *et al.*, 2019). To determine the purity of the specific rWb-SPX-1 and rWb-123 separated proteins, one gel was stained with Coomassie Brilliant Blue stain (40% methanol, 10% acetic acid and 0.25% w/v) while shaking for two hours before being de-stained in a de-staining solution (10% methanol and 7% acetic acid) while shaking at room temperature [20]. Then the gel was washed using 200 mL water and the image was recorded in Azure 280 chemiluminescent imaging system (model: Azure 280, USA)

Western blotting was done to determine the quality of the purified rWb-SXP-1 and rWb-123 proteins. The results of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were trans-blotted onto a PVDF membrane on trans-blotting buffer using a Bio-Rad Trans-Blot system at 50 mA for 30 minutes (Irumva *et al.*, 2019).

Blocking was done using blocking buffer (Blocking one, Lot# L7N3109, NacalaiTesque Inc. Kyoto Japan) on the PVDF membrane to prevent non-specific binding of the antibodies for 1 hour at room temperature with gentle shaking. The PVDF membrane was washed using 100 mL 1XTBST wash buffer while shaking at room temperature. The antigens were then incubated with diluted (1:10,000) MBL-anti His Tag HRP direct-tagged antibodies at room temperature. The PVDF membrane was then washed 5x using 100 mL of 1XTBST at room temperature while shaking for 10 minutes [20]. Excess solution was absorbed from PVDF membrane and it was incubated for five minutes at room temperature in 2 mL chemiluminescence (ECL). Then excess ECL solution was also absorbed from the PVDF membrane, and the image was captured in Azure 280 chemiluminescent imaging system (model: Azure 280, USA).

2.7. Coupling of Wb-SXP-1 and Wb-123 Proteins onto Magnetic Microspheres

The Mag-Plex Microspheres (Luminex Corporation Austin TX USA) (Irumva *et al.*, 2019) were first processed in activation buffer (0.1 M NaH_2PO_4, pH 6.2) then in1-Ethyl-3-(3-dimethylaminopropyl carbodiimide hydrochloride in the presence of Sulfo-NHS (N-hydroxysulfosuccinimide) (Lot#OI190847, Thermoscien-

tific, Rocked, USA) to generate a Sulfo-NHS-ester intermediate thus activating the carboxyl modified microspheres [20]. Using 50 Mm MES 2-(N-morpholino) ethane sulfonic, PH 5.0) coupling buffer, activated carboxyl groups on the surface of Magplex Microspheres (Luminex Corporation Austin TX USA) reacted with the primary amines of two individual rWb-SPX-1 and rWb-123 proteins forming covalent bonds through incubation for 2 hours at room temperature with gentle agitation [20] [21]. For negative control and background check beads and PBS (−) solution were used.

The beads were then washed with PBS-TBN (0.1 percent bovine serum albumin, 0.05 percent Tween 20, 0.05 percent sodium azide in PBS(−), pH 7.5) [20]. Blocking was done using PBS-TBN for 30 minutes at room temperature. Washing was done twice with PBS-TBN (0.1 percent bovine serum albumin, 0.05 percent Tween 20, 0.05 percent sodium azide in PBS (−), pH 7.5) then they were adjusted to 1000 beads per liter. A light microscope (Olympus) with a 10 objective lens was used to count the beads [22].

Wb-SXP-1 and Wb-123 Recombinant Proteins Coupling Efficiency

Antigen coupling efficiency was performed with a phycoerythrin-conjugated antibody against the poly-histidine tag to determine the number of antigens on the microspheres [22]. Briefly: 100 µL suspension of coupled beads as well as naked beads were dispensed into 96 wells plate. Then 100 µL of the first antibody MBL anti-His-tag HPR direct mouse monoclonal antibody (Code #D291-7, 22) dilute 1:1000 in staining buffer was dispensed into each well. The plate was sealed and covered with aluminum foil to prevent direct sunlight, then agitated for 30 minutes at 750 rpm on a Heidolph Titramax 100 shaker (D-91126 SchwaBach, Germany) to allow binding reaction to take place. Washing was done three times using the BIO PLEX-MAG1 program in a BioTek 405 microplate washer (Ref: 405 LBRMML, USA) [20].

100 µL of X250 diluted secondary antibody, phycoerythrin-conjugated anti-mouse IgG (Code# 710-1822, Rockland Inc.) was added, and the second binding reaction was performed at 750 rpm for 30 minutes at room temperature. Then the beads were suspended on a wash buffer and agitated at 750 rpm for 5 minutes on Heidolph Titramax 100 shaker (D-91126 SchwaBach, Germany) to allow binding reaction to take place. Washing was done three times using the BIO PLEX-MAG1 program in a Biotech washer. After washing, the beads were suspended in 125 µL of wash buffer and agitated at 750 rpm for 5 min on the shaker. Finally, the test was performed on the Bio-Plex™ 200 system (Luminex corporation Technology, USA) in normal sensitivity mode [20].

2.8. Evaluation of the Antigens Reactivity Using Multiplex Assay

The rWb-SPX-1 and rWb-123 Antigens were evaluated using the microspheres-based multiplex serological assay. Two microliters of each of the 25 *W. banchrofti* negative sera and 31 *W. banchrofti* positive sera, were diluted at a 1:50 ratio in 98 µL staining buffer (PBS-TBN (0.1% Bovine Serum albumin (BSA),

0.05% Tween 20, 0.05% sodium azide in PBS) [20]. This was dispensed into each well of a 96 well plate and 100 µL/well of rWb-SPX-1 and rWb-123 coupled proteins pulled together was added to each well [23]. The plate was covered with aluminum foil to prevent direct light and shaken at 750 rpm for 30 min at room temperature thus to enhance the binding reaction between coupled antigen and IgG antibodies in the serum samples. The plate was then be transferred to an ELx405 micro-plate washer (Bio-Tek, Winooski, VT, USA) with a magnetic plate, then washed three times using washing buffer (0.05% Tween 20, 0.05% sodium azide in PBS(2), pH 7.5) [23].

Using detection antibody Bethyl Human IgG-Fc fragment (Goat) (0.5 mg/mL) (Lot# = A80-248PE-11 Bethyl Laboratories, Inc., Montgomery, TX, USA]) diluted into 1:250 ratio in staining buffer. 100 µL was added to each well, followed by incubation of the plate for 30 min at room temperature while shaking at 750 rpm on a Heidolph Titramax 100 shaker (D-91126 SchwaBach, Germany) while covered with aluminum foil to prevent direct sunlight [22]. The plates were then washed three times using washing buffer as mentioned earlier to remove the unbound enzyme-labeled antibodies [23]. Thereafter 125 µL of wash buffer was dispensed into the wells using BioTek plate washer. The plate was sealed and covered with aluminum foil to prevent direct sunlight, then agitated for 5 minutes at 750 rpm on a Heidolph Titrama 100 shaker to allow binding reaction to take place. Finally the test was performed on the Bio-Plex 200 system (Luminex Corporation, Austin, TX, USA) in normal sensitivity mode to measure the fluorescence of the beads.

2.9. Statistical Analysis

XLSTAT 2019 (Addinsoft) was used to obtain receiver operating characteristics (ROC) analysis [20], which generated the area under the curves (AUC) that aided the description of the differences between sensitivity and specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and cut off points [24] [25] [26]. This illustrated the diagnostic ability and accuracy of the rWb-SXP-1 and rWb-123 antigens on lymphatic filariasis. Additionally box plots were as well generated using the XLSTAT program (Addinsoft) and these were used to describe samples' variability in antigens reactivity.

3. Results

3.1. Wb-SXP-1 and Wb-123 Recombinant Proteins Expression

The transformation of ECOS-BL21 (DE3) (Code#312-065334) chemically competent cells with the pET52b expression vectors encoding Wb-SXP-1 and Wb-123 DNA, respectively, was successful. Growth density of these transformed cells before the addition of Isopropyl β-D-1thigalactosidase (IPTG) was 3.75 cells/mL and 3.91 cells/mL, for Wb-SXP-1 and Wb-123 culture respectively. The supplementation of IPTG into the culture to induce protein expression thus resulted into anincreased cells growth density to 5.56 cells/mL and 6.88 cells/mL, for

Wb-SXP-1 and Wb-123 culture respectively (See Table 1).

3.2. Wb-SXP-1 and Wb-123 Recombinant Proteins Purification and Quantification

Wb-SXP-1 recombinant protein was expressed as soluble fraction while rWb-123 proteins were expressed as insoluble fraction. Both rWb-SXP-1 and rWb-123 proteins were successfully purified using Immobilized Metal Affinity Chromatography (IMAC) using TALON-Accept resin (Code#635503, Takara Bio Inc.). Quantification of these proteins at 562 nm using the Nano-drop machine indicated that rWb-SXP-1 proteins had higher concentration of 2.97 mg/mL compared to rWb-123 proteins with 1.134 mg/mL (See Table 2). Purified samples of both Wb-SXP-1 and Wb-123 recombinant antigens were analysed using 10% gel (SDS-PAGE).

The two proteins obtained presented to have molecular weights of 20.8 kDa and 70.4 kDa for rWb-SXP-1 and rWb-123 antigens respectively as shown in Figure 1. This was confirmed by western blotting with anti His Tag HRP direct-tagged antibodies and CBB.

Table 1. E. coli cells growth density before and after addition of IPTG.

Antigen	Before IPTG	After IPTG
Wb-SXP-1	3.75 cell/mL	5.56 cell/mL
Wb-123	3.91 cell/mL	6.88 cell/mL

Table 2. Protein quantification using BCA assay kit.

Sample name	Auto conc. (mg/mL)	Absorbance (562 nm)
Wb-SXP-1	2.97	2.636
Wb-123	1.134	1.087

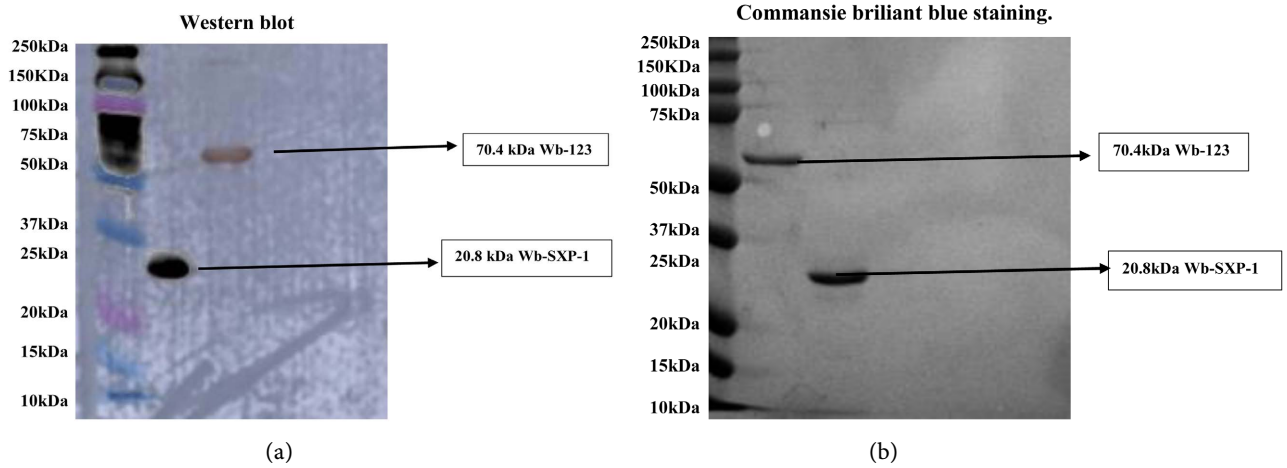


Figure 1. (a) Western Blot Analysis in 10% SDS-PAGE of the rWb-SXP-1 and rWb-123 proteins.M: Precision Marker (KDa); 1: rWb-SXP-1 protein; 2: rWb-123 protein. (b) Gel stained in CBB; M: Precision Marker (KDa); 1: rWb-SXP-1 protein; 2: rWb-123 protein.

3.3. Wb-SXP-1 and Wb-123 Recombinant Proteins Coupling and Confirmation

The amount of rWb-SXP-1 and rWb-123 antigens coupled on microsphere beads was successfully determined using a reaction with phycoerythrin-conjugated antibody against the poly-histidine tag. Based on statistical analysis results indicated that the antigens were effectively coupled on the magnetic beads. Recombinant Wb-123 antigens showing the average Median Fluorescence Intensity of 27,477 while rWb-SXP-1 antigens had an average of 16407.5 in comparison to naked beads with Median Fluorescence Intensity of 36 as its essence was for background check on the Bio-plex machine (Figure 2).

3.4. Wb-123 and Wb-SXP-1 Recombinant Antigens Immuno-Reactivity

Based on statistical analysis, Figure 3 gives a summary of the analysis conducted on the data set. Fluorescence signals for the rWb-123 and rWb-SXP-1 antigens are shown in box plots. Based on the box plots, rWb-SXP-1 antigens had higher MFI (Median Fluorescence Intensity) than Wb-123 recombinant antigen when tested against negative samples, *W. banchrofti* positive, and *B. malayi* sera samples.

In Figure 4 Wb-SXP-1 recombinant antigens presented an AUC of 0.959 (95.9% of accuracy) with 95% CI having the proportional mean between 0.913 and 1.000. While rWb-123 antigen exhibited AUC = 0.799 (79.9% of accuracy) with 95% CI having the proportional mean between 0.673 and 0.92 (see Table 3). The P-values for rW-SXP-1 and rWb-123 antigens were both obtained as P-value (<0.0001) which is less than level of significance, alpha = 0.05. Thus, the null hypothesis is rejected and we can conclude that the rWb-SXP-1 and rWb-123 antigens have diagnostic potential for lymphatic filariasis.

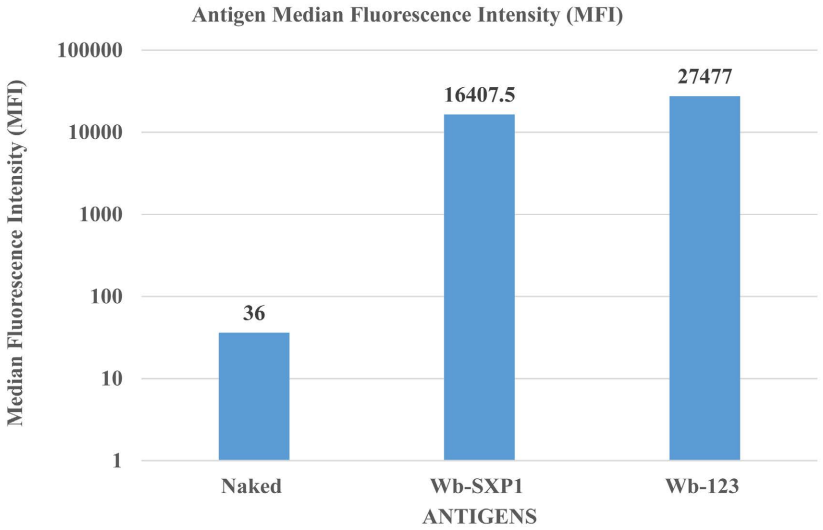
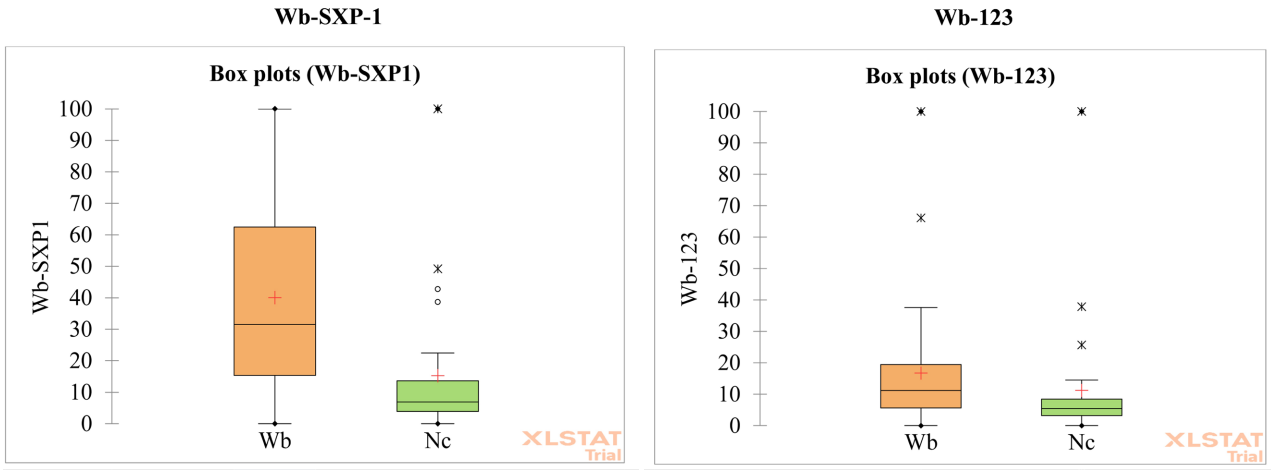
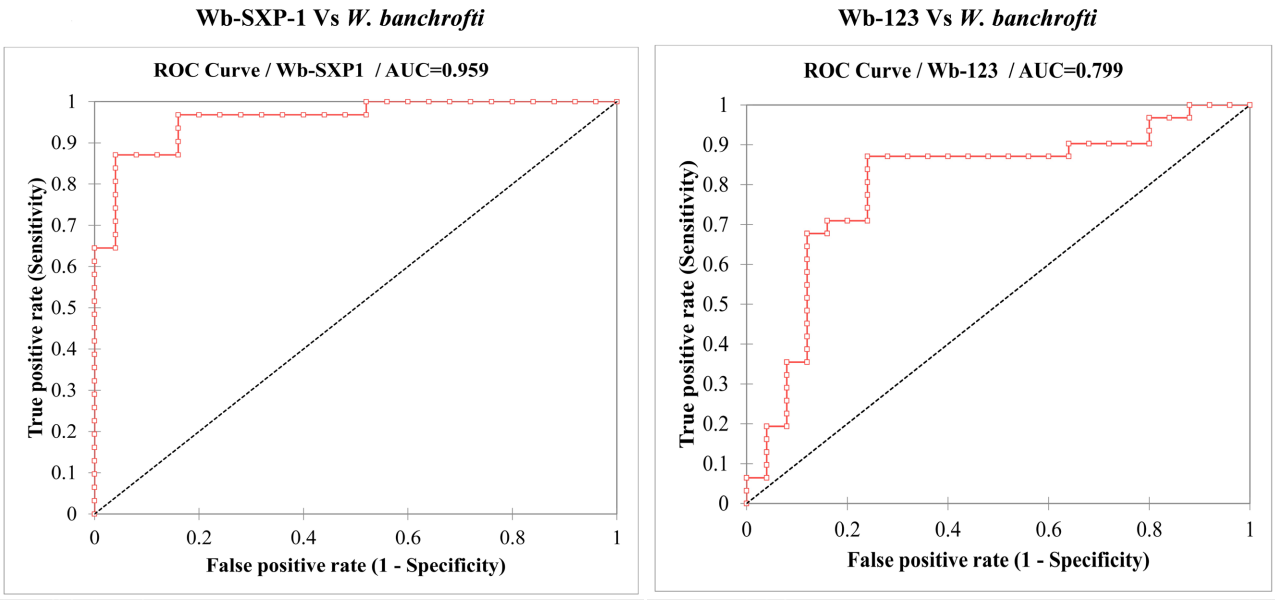


Figure 2. Bead coupling confirmation of Wb-123 and Wb-SXP-1 recombinant antigens using anti-poly-His tag antibody.



(a) (b)

Figure 3. Fluorescence signals of Wb-123 and Wb-SXP-1 recombinant antigens against negative control (NC), *W. banchrofti* (Wb), sera samples. The horizontal bars in the centre represent medians, whereas the red crosses denote means. The first and third quartiles are represented by the lower and upper box boundaries, respectively, while the box's whiskers represent the minimum and maximum values. The spots that appear outside the top and lower whisker edges identify outliers. Fold changes between infections were determined based on the medians median values.



(a) (b)

Figure 4. Receiver Operating Characteristic (ROC) curves. (a) Wb-SXP-1 recombinant antigens tested against *Wuchereria banchrofti* positive sera samples. (b) Wb-123 recombinant antigens tested against *Wuchereria banchrofti* positive sera samples.

Table 3. Sensitivity, specificity, PPV, NPV and Area under the Curve (AUC) of Wb-SXP-1 and Wb-123 recombinant antigens summary.

Antigens	Infection	Sensitivity	95% CI		Specificity	95% CI		AUC	PPV	NPV	Cut-off
			Lower	Upper		Lower	Upper				
Wb-123	*W. banchrofti*	0.871	0.704	0.953	0.760	0.561	0.887	0.799	0.818	0.826	968
WbSXP-1	*W. banchrofti*	0.871	0.704	0.953	0.960	0.786	1.000	0.959	0.964	0.857	2714

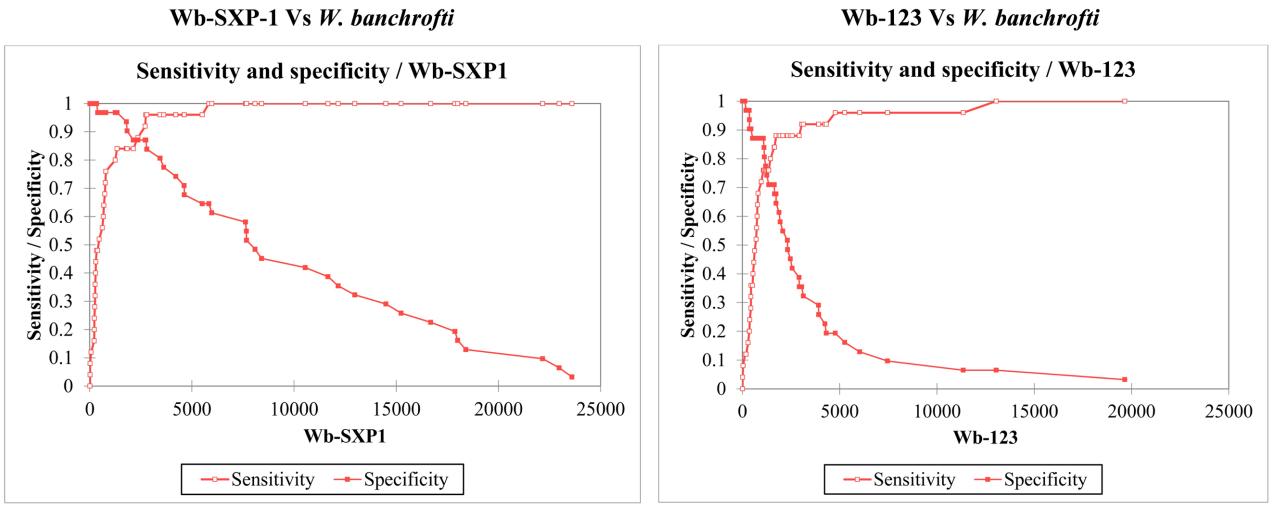


Figure 5. Immuno-reactivity curves. (a) Wb-SXP-1 recombinant antigens sensitivity and specificity to *W. banchrofti* positive sera samples. (b) Wb-123 recombinant antigens sensitivity and specificity to *W. banchrofti* positive sera samples.

Wb-SXP-1 recombinant antigens were able to distinguish between positive and negative samples for *W. banchrofti* serum with 2714 as optimal cut-off, having 87.1% sensitivity and 96.0% specificity with PPV and NPV of 96.4% and 85.7% respectively (see **Table 3**). While, Wb-123 antigens were able to distinguish between positive and negative samples for *W. banchrofti* serum with 968 as optimal cut-off, having 87.1% sensitivity and 76.0% specificity with PPV of 81.8% and NPV of 82.6% (see **Table 3**). However, rWb-SXP-1 antigens presented a higher specificity of 96.0% to *W. banchrofti* positive serum samples compared to 76.0% specificity of Wb-123 antigens (**Figure 5**).

4. Discussion

To enable progress toward achieving the Sustainable Development Goal (SDG) of 2030, in accordance with the WHO road-map towards the total worldwide elimination of neglected tropical diseases [27], acquiring reliable diagnostic methods that are specific, sensitive rapid and relatively inexpensive to perform on large numbers of sample capable of supporting the program to success is of great importance [15]. The use of antibody-based tests to identify exposure to filarial parasites has gained attention as diagnostic techniques, providing possible early warning signals of *W. banchrofti* transmission [28] [29]. Parallel to results of previous studies [20] [21], *W. banchrofti* antigens, Wb-123 and Wb-SXP-1 recombinant plasmids were successfully transformed into Escherichia coli strain BL21 (DE3) and the genes were induced to express the specific proteins by the supplementation of Isoproplyl-β-d-1-thiogalactopyranoside (IPTG) to the culture. Binding of IPTG to the lac operon, releasing the lac operator in an allosteric manner thus allowing the transcription of the specific Wb-123 and Wb-SXP-1 antigens, was evidenced by the increase of Cells growth density increased to 5.56

cells/mL (rWb SXP-1 antigens) and 6.88 cells/mLμ (rWb-123 antigens) when compared to cells growth density before the addition of IPTG. Protein extraction resulted to Wb-SXP-1 proteins as soluble fraction while rWb-123 proteins as insoluble fraction had presence of inclusion bodies. Previous studies [30] [31] [32] suggest that insolubility of rWb-123 expressed proteins could be as a result of IPTG inducer concentration being high and presence of strong promoter systems that might resulted to expression of the rWb-123 proteins at a high translational rate, exhausting bacterial protein quality control system and the partially folded and misfolded protein molecules aggregated to form inclusion bodies.Purification of these proteins using immobilized metal affinity chromatograph (IMAC) parallel to previous studies [33] [34] was successful with the purpose of preventing non-specific binding of antibodies other that those specifically synthesized against *W. banchrofti*. This was confirmed through BSA protein quantification where protein rWb-SXP-1 proteins had higher concentration of 2.97 mg/mL compared to rWb-123 proteins with a protein concentration amount of 1.134 mg/mL a result likely to have been contributed by inefficient recovery proteins from the inclusion bodies. Similar to studies done earlier [34] [35], separation of rWb-SXP-1 and rWb-123 proteins through SDS PAGE yielded molecular weights of 20.8 kDa and 70.4 kDa respectively and purity and quality levels was confirmed through western blot and Coomassie brilliant blue staining.

The multiplex assay technique is based on the same theoretical ideas as the ELISA technology and the interpretation of the results of the microsphere-based multiplex serological assays is comparable to that of the ELISA results [21]. Nevertheless, compared to ELISA, the multiplex assay technique allows researchers to examine numerous analytes concurrently, yielding more data from a smaller sample volume in a shorter amount of time [36]. Utilizing *W. banchrofti* sera (two microliters), microsphere-based multiplex technology evaluation of Wb-SXP-1 and Wb-123 recombinant antigens was done simultaneously and results were obtained within a short period. These findings are consistent with past studies in which numerous antigens were assessed utilizing multiplex technology against several pathogens, demonstrating advances in serological detection such as speed, repeatability, and need for fewer samples [37] [38] [39].

Based on statistical analysis of data generated from multiplex technology assay, generated ROC curves for both rWb-SXP-1 and rWb-123 antigens against *W. banchrofti*, indicated that the two antigens are capable of detecting immunoglobulin G4 (IgG4) antibodies in human sera synthesized specifically against *W. banchrofti* infections. Both the rWb-SXP-1 and rWb-123 antigens assay revealed 87.1% sensitivity to serum from *W. banchrofti* patients an outcome that was lower when compared to previous investigation outcomes where ELISA and Rapid tests were used and sensitivity was found to be greater than 90% [35] [37] [40] [41]. However, despite the fact that both antigens had diagnostic capabilities of *W. banchrofti* for LF diagnosis, when AUC values from ROC analysis were

considered, Wb-SXP-1 antigens yielded an AUC value of 0.959, indicating a 95.9% chance of correctly distinguishing between positive and negative samples of *W. banchrofti* with a higher specificity of 96%, PPV of 96.7%, and NPV of 85. This was in contrast to Wb-123 recombinant antigens, which had a lower AUC value of 0.799, specificity of 76%, PPV of 81.8%, and NPV of 82.6%. Hence, Wb-SXP-1 recombinant antigens were demonstrated to be of higher specificity than Wb-123 recombinant antigens when used for diagnosis of *W. banchrofti* in human sera.

5. Conclusion

Based on the immune-reactivity reaction of rWb-SXP-1 and rWb-123 antigens, results indicated that *W. banchrofti* infections can be detected by antibody profiling with excellent diagnostic sensitivity and specificity using microsphere-based multiplex serological tests. To improve the monitoring and assessment of lymphatic filariasis infections in endemic areas, rWb123 and rWb-SXP-1 microsphere-based multiplex serological tests can be particularly practical for screening a large number of sera samples and/or for quick, extensive field-testing due to the high-throughput, quick formats applied.

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Authors' Contributions

Conceptualization, S.M.N.; Methodology, S.K.M., S.M.N., R.W.W., A.W.M., and P.K.R.; Software, S.K.M.; Validation, S.M.N., R.W.W., A.W.M., and R.M.I.; Formal Analysis, S.K.M., S.M.N., and A.W.M.; Investigation, S.K.M., A.W.M., S.M.N., M.M.M., R.M.I., C.W.N., P.K.R., T.T.N., J.J.Y., G.N.K., N.S.T., P.M.N. and D.K.O.; Resources, S.M.N.; Data Curation, S.M.N. and S.K.M.; Writing-Original Draft Preparation, S.K.M., Writing-Review and Editing, S.M.N., R.W.W and R.M.I.; Visualization, S.M.N., A.W.M. and R.W.W.; Supervision, S.M.N. and R.W.W.; Project Administration, S.M.N. and R.W.W.; Funding Acquisition, S.M.N.

Institutional Review Board Statement

The JKUAT-IERC approved this study under reference number JKU/IERC/02316/0586. The samples used in this study were obtained from the Pan African

Hub of Infectious Diseases Laboratory based at KEMRI headquarters in Nairobi, Kenya.

Data Availability Statement

The data presented in this study are available in form of figures, supplementary figures, and tables provided within the article.

Conflicts of Interest

The authors declare that there are no competing interests.

References

[1] Allen, S.P., Polazzi, J.O., Gierse, J.K. and Easton, A.M. (1992) Two Novel Heat Shock Genes Encoding Proteins Produced in Response to Heterologous Protein Expression in *Escherichia coli*. *Journal of Bacteriology*, **174**, 6938-6947. https://doi.org/10.1128/jb.174.21.6938-6947.1992

[2] Baskar, L.K.V., Srikanth, T.R., Suba, S., Mody, H.C., Desai, P.K. and Kaliraj, P. (2004) Development and Evaluation of a Rapid Flow-Through Immunofiltration Test Using Recombinant Filarial Antigen for Diagnosis of Brugian and Bancroftian Filariasis. *Microbiology and Immunology*, **48**, 519-525. https://doi.org/10.1111/j.1348-0421.2004.tb03547.x

[3] Bukau, B. (1993) Regulation of the *Escherichia coli* Heat-Shock Response. *Molecular Microbiology*, **9**, 671-680. https://doi.org/10.1111/j.1365-2958.1993.tb01727.x

[4] Cadavid Restrepo, A.M., Gass, K., Won, K.Y., Sheel, M., Robinson, K., Graves, P.M., Fuimaono, S. and Lau, C.L. (2022) Potential Use of Antibodies to Provide an Earlier Indication of Lymphatic Filariasis Resurgence in Post-Mass Drug Administration Surveillance in American Samoa. *International Journal of Infectious Diseases*, **117**, 378-386. https://doi.org/10.1016/j.ijid.2022.02.006

[5] Carrió, M.M. and Villaverde, A. (2005) Localization of Chaperones DnaK and GroEL in Bacterial Inclusion Bodies. *Journal of Bacteriology*, **187**, 3599-3601. https://doi.org/10.1128/JB.187.10.3599-3601.2005

[6] Carter, J.V., Pan, J., Rai, S.N. and Galandiuk, S. (2016) ROC-ing along: Evaluation and Interpretation of Receiver Operating Characteristic Curves. *Surgery* (*United States*), **159**, 1638-1645. https://doi.org/10.1016/j.surg.2015.12.029

[7] Chandy, A., Thakur, A.S., Singh, M.P. and Manigauha, A. (2011) A Review of Neglected Tropical Diseases: Filariasis. *Asian Pacific Journal of Tropical Medicine*, **4**, 581-586. https://doi.org/10.1016/S1995-7645(11)60150-8

[8] Fujii, Y., Kaneko, S., Nzou, S. M., Mwau, M., Njenga, S.M., Tanigawa, C., Kimotho, J., Mwangi, A.W., Kiche, I. and Matsumoto, S. (2014) Serological Surveillance Development for Tropical Infectious Diseases Using Simultaneous Microsphere-Based Multiplex Assays and Finite Mixture Models. *PLOS Neglected Tropical Diseases*, **8**, e3040. https://doi.org/10.1371/journal.pntd.0003040

[9] Gan, S.D. and Patel, K.R. (2013) Enzyme Immunoassay and Enzyme-Linked Immunosorbent Assay. *Journal of Investigative Dermatology*, **133**, 1-3. https://doi.org/10.1038/jid.2013.287

[10] Ganapathy, M., Chakravarthi, M., Charles, S.J., Harunipriya, P., Jaiganesh, S., Subramonian, N. and Kaliraj, P. (2015) Immunodiagnostic Properties of *Wuchereria bancrofti* SXP-1, a Potential Filarial Diagnostic Candidate Expressed in Tobacco

Plant, *Nicotiana tabacum*. *Applied Biochemistry and Biotechnology*, **176**, 1889-1903. https://doi.org/10.1007/s12010-015-1685-0

[11] Gass, K., de Rochars, M.V.E.B., Boakye, D., Bradley, M., Fischer, P.U., Gyapong, J., Itoh, M., Ituaso-Conway, N., Joseph, H., Kyelem, D., Laney, S.J., Legrand, A.M., Liyanage, T.S., Melrose, W., Mohammed, K., Pilotte, N., Ottesen, E.A., Plichart, C., Ramaiah, K. and Lammie, P. (2012) A Multicenter Evaluation of Diagnostic Tools to Define Endpoints for Programs to Eliminate Bancroftian Filariasis. *PLOS Neglected Tropical Diseases*, **6**, e1479. https://doi.org/10.1371/journal.pntd.0001479

[12] Gounoue-kamkumo, R., Nana-djeunga, H.C., Bopda, J., Akame, J., Tarini, A. and Kamgno, J. (2015) Loss of Sensitivity of Immunochromatographic Test (ICT) for Lymphatic Filariasis Diagnosis in Low Prevalence Settings: Consequence in the Monitoring and Evaluation Procedures. *BMC Infectious Diseases*, **15**, Article No. 579. https://doi.org/10.1186/s12879-015-1317-x

[13] Houser, B. (2012) Bio-rad's Bio-Plex® Suspension Array System, xMAP Technology Overview. *Archives of Physiology and Biochemistry*, **118**, 192-196. https://doi.org/10.3109/13813455.2012.705301

[14] Irumva, V., Waihenya, R., Mwangi, A.W., Kipkemboi, P., Kaneko, S., Teya, T., Jepkemei, J., Njoroge, C., Gitari, J.W., Munyao, M.M., Irekwa, R.M., Ng'ang'a, M. and Nzou, S.M. (2019) Evaluation of the TbgI2 and TbgI17 Tandem Repeat Antigens as Potential Antigens for the Diagnosis of *Trypanosoma brucei rhodesiense*. *Open Journal of Clinical Diagnostics*, **9**, 152-163. https://doi.org/10.4236/ojcd.2019.94011

[15] Kubofcik, J., Fink, D.L. and Nutman, T.B. (2012) Identification of Wb123 as an Early and Specific Marker of *Wuchereria bancrofti* Infection. *PLOS Neglected Tropical Diseases*, **6**, e1930. https://doi.org/10.1371/journal.pntd.0001930

[16] Kumar, R. and Indrayan, A. (2011) Kumar-2011-Receiver Operating Characteristic 1. *Indian Pediatrics*, **48**, 277-287. https://doi.org/10.1007/s13312-011-0055-4

[17] Lammie, P.J., Weil, G., Noordin, R., Kaliraj, P., Steel, C., Goodman, D., Lakshmikanthan, V.B. and Ottensen, E. (2004) Recombinant Antigen-Based Antibody Assays for the Diagnosis and Surveillance of Lymphatic Filariasis—A Multicenter Trial. *Filaria Journal*, **3**, Article No. 9. https://doi.org/10.1186/1475-2883-3-9

[18] Malecela, M.N. and Ducker, C. (2021) A Road Map for Neglected Tropical Diseases 2021-2030. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **115**, 121-123. https://doi.org/10.1093/trstmh/trab002

[19] Mandrekar, J.N. (2010) Receiver Operating Characteristic Curve in Diagnostic Test Assessment. *Journal of Thoracic Oncology*, **5**, 1315-1316. https://doi.org/10.1097/JTO.0b013e3181ec173d

[20] Nzou, S.M., Fujii, Y., Miura, M., Mwau, M., Mwangi, A. W., Itoh, M., Salam, M.A., Hamano, S., Hirayama, K. and Kaneko, S. (2016) Development of Multiplex Serological Assay for the Detection of Human African Trypanosomiasis. *Parasitology International*, **65**, 121-127. https://doi.org/10.1016/j.parint.2015.10.008

[21] Rahmah, N., Lim, B.H., Khairul Anuar, A., Shenoy, R.K., Kumaraswami, V., Lokman Hakim, S., Chotechuang, P., Kanjanopas, K. and Ramachandran, C.P. (2001) A Recombinant Antigen-Based IgG4 ELISA for the Specific and Sensitive Detection of *Brugia malayi* Infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95**, 280-284. https://doi.org/10.1016/S0035-9203(01)90234-2

[22] Ramaiah, K.D., Das, P.K., Michael, E. and Guyatt, H.L. (2000) The Economic Burden of Lymphatic Filariasis in India. *Parasitology Today*, **16**, 251-253. https://doi.org/10.1016/S0169-4758(00)01643-4

[23] Rao, K.V.N., Eswaran, M., Ravi, V., Gnanasekhar, B., Narayanan, R.B., Kaliraj, P.,

Jayaraman, K., Marson, A., Raghavan, N. and Scott, A.L. (2000) The *Wuchereria bancrofti orthologue* of *Brugia malayi* SXP1 and the Diagnosis of Bancroftian Filariasis. *Molecular and Biochemical Parasitology*, **107**, 71-80. https://doi.org/10.1016/S0166-6851(99)00231-5

[24] Rebollo, M.P. and Bockarie, M.J. (2014) Shrinking the Lymphatic Filariasis Map: Update on Diagnostic Tools for Mapping and Transmission Monitoring. *Parasitology*, **141**, 1912-1917. https://doi.org/10.1017/S0031182014001231

[25] Restrepo, C., Gass, K., Won, K., Sheel, M., Robinson, K., Graves, P., Fuimaono, S. and Lau, C. (2022) Potential Use of Antibodies to Provide an Earlier Indication of Lymphatic Filariasis Resurgence in Post-Mass Drug Administration Surveillance in American Samoa. *International Journal of Infectious Diseases*, **117**, 378-386. https://doi.org/10.1016/j.ijid.2022.02.006

[26] Sarr, J., Orlandi-Pradines, E., Fortin, S., Sow, C., Cornelie, S., Rogerie, F., Guindo, S., Konate, L., Fusa, T., Riveau, G., Rogier, C. and Remoue, F. (2011) Assessment of Exposure to *Plasmodium falciparum* Transmission in a Low Endemicity Area by Using Multiplex Fluorescent Microsphere-Based Serological Assays. *Parasites and Vectors*, **4**, Article No. 212. https://doi.org/10.1186/1756-3305-4-212

[27] Solomon, A.W., Engels, D., Bailey, R.L., Blake, I.M., Brooker, S., Chen, J.X., Chen, J.H., Churcher, T.S., Drakeley, C.J., Edwards, T., Fenwick, A., French, M., Gabrielli, A.F., Grassly, N.C., Harding-Esch, E.M., Holland, M.J., Koukounari, A., Lammie, P.J., Leslie, J. and Peeling, R.W. (2012) A Diagnostics Platform for the Integrated Mapping, Monitoring, and Surveillance of Neglected Tropical Diseases: Rationale and Target Product Profiles. *PLOS Neglected Tropical Diseases*, **6**, e1746. https://doi.org/10.1371/journal.pntd.0001746

[28] Steel, C., Golden, A., Kubofcik, J., LaRue, N., De los Santos, T., Domingo, G.J. and Nutman, T.B. (2013) Rapid *Wuchereria bancrofti*-Specific Antigen Wb123-Based IgG4 Immunoassays as Tools for Surveillance Following Mass Drug Administration Programs on Lymphatic Filariasis. *Clinical and Vaccine Immunology*, **20**, 1155-1161. https://doi.org/10.1128/CVI.00252-13

[29] Steel, C., Golden, A., Stevens, E., Yokobe, L., Domingo, G.J., De los Santos, T. and Nutman, T.B. (2015) Rapid Point-of-Contact Tool for Mapping and Integrated Surveillance of *Wuchereria bancrofti* and *Onchocerca volvulus* Infection. *Clinical and Vaccine Immunology*, **22**, 896-901. https://doi.org/10.1128/CVI.00227-15

[30] Tanigawa, C., Fujii, Y., Miura, M. and Nzou, S.M. (2015) Species-Specific Serological Detection for Schistosomiasis by Serine Protease Inhibitor (SERPIN) in Multiplex Assay. *PLOS Neglected Tropical Diseases*, **9**, e0004021. https://doi.org/10.1371/journal.pntd.0004021

[31] Taylor, M.J., Hoerauf, A. and Bockarie, M. (2010) Lymphatic Filariasis and Onchocerciasis. *The Lancet*, **376**, 1175-1185. https://doi.org/10.1016/S0140-6736(10)60586-7

[32] Janardhan, S., *et al.* (2007) Production, Purification and Diagnostic Application of Filarial Recombinant Protein WbSXP-1 Expressed in Salt Inducible *Escherichia coli*. *Journal of Industrial Microbiology and Biotechnology*, **34**, 675-683. https://doi.org/10.1007/s10295-007-0243-3

[33] Tyrell, E. (2013) Socioeconomic Burden of Lymphatic Filariasis in Georgetown, Guyana. *Tropical Medicine and International Health*, **18**, 152-158. https://doi.org/10.1111/tmi.12017

[34] Wanji, S., Amvongo-Adjia, N., Koudou, B., Njouendou, A.J., Chounna Ndongmo, P.W., Kengne-Ouafo, J.A., Datchoua-Poutcheu, F.R., Fovennso, B.A., Tayong, D.B.,

Fombad, F.F., Fischer, P.U., Enyong, P.I. and Bockarie, M. (2015) Cross-Reactivity of Filariais ICT Cards in Areas of Contrasting Endemicity of *Loa loa* and *Mansonella perstans* in Cameroon: Implications for Shrinking of the Lymphatic Filariasis Map in the Central African Region. *PLOS Neglected Tropical Diseases*, **9**, e0004184. https://doi.org/10.1371/journal.pntd.0004184

[35] Wanji, S., Amvongo-Adjia, N., Njouendou, A.J., Kengne-Ouafo, J.A., Ndongmo, W. P.C., Fombad, F.F., Koudou, B., Enyong, P.A. and Bockarie, M. (2016) Further Evidence of the Cross-Reactivity of the Binax NOW® Filariasis ICT Cards to Non-*Wuchereria bancrofti filariae*: Experimental Studies with *Loa loa* and *Onchocerca ochengi*. *Parasites and Vectors*, **9**, Article No. 267. https://doi.org/10.1186/s13071-016-1556-8

[36] Weil, G.J. and Ramzy, R.M.R. (2007) Diagnostic Tools for Filariasis Elimination Programs. *Trends in Parasitology*, **23**, 78-82. https://doi.org/10.1016/j.pt.2006.12.001

[37] Won, K., Gass, K., Biamonte, M., Dagne, D.A., Ducker, C., Hanna, C., Hoerauf, A., Lammie, P., Njenga, S., Noordin, R., Ramaiah, K., Ramzy, R., Scholte, R.G.C., Solomon, A., Souza, A., Tappero, J., Toubali, E., Weil, G., Williams, S. and King, J. (2021) Diagnostics to Support Elimination of Lymphatic Filariasis-Development of Two Target Product Profiles. *PLOS Neglected Tropical Diseases*, **15**, e0009968. https://doi.org/10.1371/journal.pntd.0009968

[38] Won, K., Sambou, S., Barry, A., Robinson, K., Jaye, M., Sanneh, B., Sanyang, A., Gass, K., Lammie, P. and Rebollo, M. (2018) Use of Antibody Tools to Provide Serologic Evidence of Elimination of Lymphatic Filariasis in the Gambia. *American Journal of Tropical Medicine and Hygiene*, **98**, 15-20. https://doi.org/10.4269/ajtmh.17-0371

[39] World Health Organization (WHO) (n.d.) Control of Neglected Tropical Diseases. https://www.who.int/teams/control-of-neglected-tropical-diseases/lymphatic-filariasis/diagnosis-and-treatment

[40] World Health Organization (WHO) (n.d.) Lymphatic Filariasis. https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis

[41] Zeldenryk, L.M., Gray, M., Speare, R., Gordon, S. and Melrose, W. (2011) The Emerging Story of Disability Associated with Lymphatic Filariasis: A Critical Review. *PLOS Neglected Tropical Diseases*, **5**, e1366. https://doi.org/10.1371/journal.pntd.0001366