Biomarker discovery to detect antibodies specific for *Wuchereria bancrofti*

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BACKGROUND

BACKGROUND

The World Health Organization (WHO) neglected tropical diseases road map for 2021–2030 calls for better diagnostic tests that would allow greater precision in program delivery. Tests are needed both for diagnosing active infections and detecting recrudescence during the post-MDA surveillance phase. To address this, we sought to identify new antibody biomarkers that are sensitive and specific for bancroftian lymphatic filariasis (LF).

OBJECTIVES

➢To identify antibody biomarkers for bancroftian LF that are not cross-reacted to onchocerciasis-positive sera.

➢To develop an antibody detection assay for LF that could be used for post-MDA surveillance.

METHODS

1. SERA

SERA (serum epitope reservoir analysis) incorporates a large random peptide library, Next Generation Sequencing (NGS), and custom bioinformatics tools to map immunogenic antigen epitopes targeted for a given infection.

An antibody-containing patient sample (typically serum or plasma) is incubated with a bacterial library displaying random peptides at the cell surface. Antibodies bind to peptide library members that mimic the binding site on their natural protein target. Antibody (IgG total, IgG1, and IgG4 subclasses) bound bacteria are magnetically separated to isolate a unique set of antibody-binding peptides for each patient. NGS is used to sequence each patient's set of millions of antibody-binding peptides (i.e. each patient's antibody epitope repertoires). From these peptide datasets, custom computer algorithms identify amino acid motifs that occur in the diseased patient samples and are absent from controls. Motifs are aligned to proteome sequences of interest to identify likely antigen candidates eliciting the patient's antibody response. The most sensitive and specific motifs and antigens are identified for the disease cohort compared to controls and additional databased samples. A validation cohort of samples is then used to evaluate the performance of the selected motifs and antigens. The best-performing antigens are candidates for testing using a simple diagnostic platform such as ELISA or lateral flow (Kamath, K, et al. Sci Rep. 10, 5294 (2020); Reifert, J, et al. J Clin Microbiol. 59, e01836-20 (2021); Pantazes, RJ, et al. Sci Rep. 6, 30312 (2016); Haynes, WA, Kamath, K, Wait,z R, Daugherty, PS, Shon, JC. Front Immunol. 12, 625311 (2021)).

2. Peptide Array

The protein sequences of *W. bancrofti* and *O. volvulus* from SERA platform and from secretomes and glycosyl-phosphatidyl inositol (GPI)-anchored proteins were converted into a lymphatic filariasis peptide microarray. Peptides with a length of >15 amino acids were converted into 15 amino acid peptides with a peptide-peptide overlap of 13 amino acids. The resulting lymphatic filariasis peptide microarrays contained 4,879 different peptides printed in duplicate (9,758 peptide spots) and were framed by additional HA (YPYDVPDYAG, 108 spots) and polio (KEVPALTAVETGAT, 108 spots) control peptides. The slides were washed using PBS, pH 7.4 with 0.05% Tween 20 for 3 x 10 sec followed by a 30-minutes blocking step in Rockland blocking buffer MB-070. The slides were then exposed to *W. bancrofti* positive, Wuchereria bancrofti negative (from non-endemic countries), *O. volvulus* positive from *W. bancrofti* non-endemic area, and *Brugia malayi* positive sera diluted 1:200 (IgG total and IgG4) and 1:100 (IgG1) in 10% blocking buffer. Sera incubation was carried out for 16 h at 4°C and orbital shaking at 140 rpm. After washing as above, goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml), mouse anti-human IgG4 DyLight800 (0.2 µg/ml), and mouse anti-human IgG1 Cy3 (0.2 µg/ml) were added for 45 minutes staining in the blocking buffer at RT. The fluorescence was read by a scanner (LI-COR Odyssey Imaging System) with scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm/green = 800 nm) and Genepix Imaging System, resolution 10 µm, gain of 500 PMT(red = 632 nm / green = 532 nm)

3. Peptide ELISA

Peptides identified through the SERA platform and peptides that showed significant signal-to-noise ratios in the peptide array were synthesized using the service of a commercial company. After peptide resuspension to the respective diluent at 2 mg/mL, peptides were diluted to 10 ug/mL in sensitization buffer (50 mM Tris-HCl, ph 8.0 + 0.3 M KCl + 2 mM EDTA) and added to wells and then peptide sensitization was carried out at 4 C overnight.

The plate was washed with PBS/0.3% Tween-20 and then diluted serum (1:50 in PBS/0.3% Tween-20/5% milk) was added to the wells. After 30 minutes of incubation, the plate was washed and secondary antibodies (either IgG total, IgG1, or IgG4) – HRP conjugated was added. After a further 30 minutes of incubation, the plate was washed, and TMB substrate was added. The reaction was observed at A650 nm.

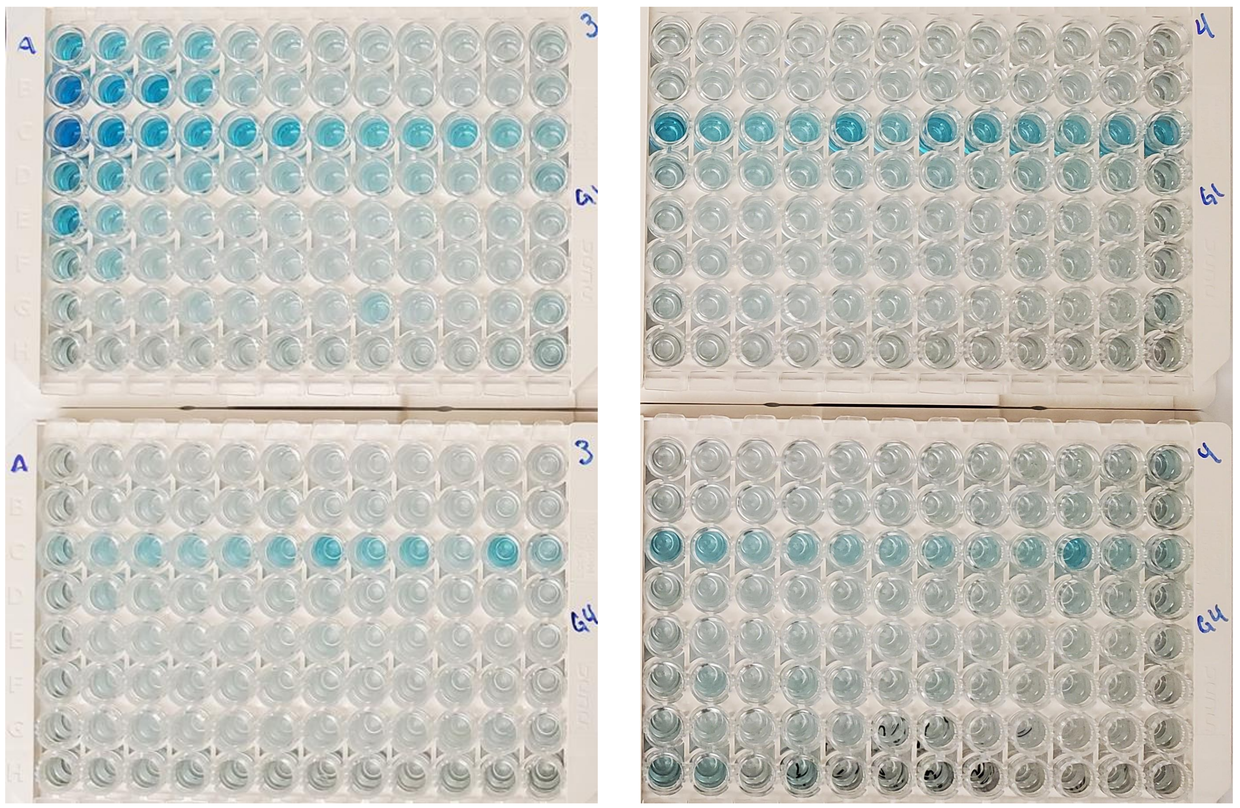

Figure 1: ELISA results of peptide array. Peptides were tested against IgG1 and IgG4.

IgG1	2	3	5	7	8	9	12	15	16	17	18	19
Negative	0.464933	0.1321	0.187933	0.0505	0.016067	0.009767	0.0082	0.0348	0.000333	0.026133	0.00185	0.000733
Wb	1.433933	0.7421	0.4563	0.359933	0.431	0.4375	0.268	0.239333	0.2261	0.3164	0.1618	0.0874
Ov	0.175933	0.1472	0.015	0.078733	0.0406	0.0586	0.0458	0.076233	0.0493	0.0599	0.0379	0.072
S/N Wb	3.1	5.6	2.4	7.1	26.8	44.8	32.7	6.9	678.3	12.1	87.5	119.2
S/N Ov	0.4	1.1	0.1	1.6	2.5	6.0	5.6	2.2	147.9	2.3	20.5	98.2
S/N Wb/O	8.2	5.0	30.4	4.6	10.6	7.5	5.9	3.1	4.6	5.3	4.3	1.2
IgG4	2	3	5	7	8	9	12	15	16	17	18	19
Negative	0.0026	0.0052	0.0127	0.0014	0.003033	0.005567	0.010933	0.003467	0.009567	0.00185	0.0075	0.0048
Wb	0.07545	0.1184	0.156333	0.1157	0.149067	0.203667	0.313733	0.203533	0.2234	0.05915	0.2964	0.114967
Ov	0.00415	0.0602	0.004533	0.0111	-0.00513	0.007767	0.006933	0.004433	0.0389	-0.00055	0.0065	0.010567
S/N Wb	29.0	22.8	12.3	82.6	49.1	36.6	28.7	58.7	23.4	32.0	39.5	24.0
S/N Ov	1.6	11.6	0.4	7.9	-1.7	1.4	0.6	1.3	4.1	-0.3	0.9	2.2
S/N Wb/O	18.2	2.0	34.5	10.4	-29.0	26.2	45.3	45.9	5.7	-107.5	45.6	10.9

IgG1	20	21	22	23	24	27	28	29	30	31	32	33
Negative	0.010867	0.001117	0.0151	0.0149	0.0018	0.016	0.0016	0.003683	0.008733	0.003	0.0053	0.0298
Wb	0.4724	0.25235	0.1989	0.1931	0.406467	0.164333	0.412033	0.32825	0.281167	0.193933	0.3053	0.395333
Ov	0.0386	0.02105	0.0563	0.0627	0.038467	0.033633	0.055933	0.03355	0.058967	0.052733	0.0417	0.039033
S/N Wb	43.5	226.0	13.2	13.0	225.8	10.3	257.5	89.1	32.2	64.6	57.6	13.3
S/N Ov	3.6	18.9	3.7	4.2	21.4	2.1	35.0	9.1	6.8	17.6	7.9	1.3
S/N Wb/O	12.2	12.0	3.5	3.1	10.6	4.9	7.4	9.8	4.8	3.7	7.3	10.1
IgG4	20	21	22	23	24	27	28	29	30	31	32	33
Negative	0.010133	0.0063	0.012067	0.018833	0.000633	0.006867	0.006667	0.021367	0.006567	0.000333	0.0026	0.018233
Wb	0.2178	0.1999	0.11	0.1371	0.1019	0.102733	0.1061	0.107233	0.104267	0.3164	0.0969	0.1058
Ov	0.0103	0.013	0.0127	0.0137	0.0021	0.008333	0.0037	0.026733	0.001867	0.0306	0.01	0.0252
S/N Wb	21.5	31.7	9.1	7.3	160.9	15.0	15.9	5.0	15.9	949.2	37.3	5.8
S/N Ov	1.0	2.1	1.1	0.7	3.3	1.2	0.6	1.3	0.3	91.8	3.8	1.4
S/N Wb/O	21.1	15.4	8.7	10.0	48.5	12.3	28.7	4.0	55.9	10.3	9.7	4.2

Figure 2a and 2b: Signal-to-noise (S/N) calculations for Plate 1-IgG1 and IgG4 (top) and Plate 2-IgG1 and IgG4 (bottom).

RESULTS

➢SERA Results

➢60 defined bancroftian LF (infected – antigen and microfilariae positive and exposed – negative for LF antigen and microfilariae) from Haiti, 60 defined onchocerciasis negative sera, and 60 defined onchocerciasis positive sera from Guatemala were subjected to the SERA platform.

➢There are 49 specific epitopes for IgG total, 93 epitopes for IgG1, and 28 epitopes for IgG4 for LF infected or exposed defined sera while there are 8 specific epitopes for IgG total, 5 epitopes for IgG1, and 96 epitopes for IgG4.

➢These epitopes and additional peptides identified through secretome, and GPI-anchored proteins were further screened using a peptide array. Based on significant signal-to-noise results (S/N > 10), we synthesized 99 peptide sequences for further screening in peptide ELISA.

➢Peptide Array Results

The ELISA results can be seen in Figure 1. Based on the criteria of S/N W. bancrofti to negative > 10 and S/N W. bancrofti to O. volvulus > 5, we identified 22 peptides for detection of IgG1 antibody responses and W. bancrofti infection and 21 peptides for detection of IgG4 antibody. It seems that IgG4 antibody responses yield a better S/N ratio than IgG1 antibody responses.

DISCUSSION

While the peptide ELISA gave several potential peptides for the detection of IgG1 and IgG4 antibody responses to *W. bancrofti* infection, there is a possibility that some peptides did not bind well to the well of the ELISA plate.

Before we conclude the study on antibody biomarkers for *W. bancrofti* infection, we need to use a potentially better binding of peptide to the well by biotinylating of peptides and adding them to the streptavidin plate.

CONCLUSION AND NEXT STEPS

SERA platform, peptide array, and peptide ELISA identified several potential peptides for the detection of IgG1 and IgG4 antibody responses to *W. bancrofti* infection in less than 5 months.

Conduct biotinylated peptide ELISA on a streptavidin plate.

Significant peptides will be used to produce a chimeric protein containing several peptides.

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Biomarker Discovery and Assay Development to Detect Antibodies to *Onchocerca volvulus* based on OvCol-1 and Ov7

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BACKGROUND

Onchocerciasis affects ~21 million people, with 99% of cases reported in 31 sub-Saharan countries. It is a high burden disease and is therefore targeted for elimination. The control and elimination programs of the disease is primarily based on mass-drug administration (MDA) using the anti-helminthic drug ivermectin. The elimination program strategy is a multi-phase process. Due to the differences in control or elimination stages, tools needed to support the program are needed. The tools needed for monitoring and evaluation are mostly based on detecting antibodies in the population. The World Health Organization (WHO) neglected tropical diseases road map for 2021-2030 highlighted the need for better diagnostic tests to support control and elimination of onchocerciasis. WHO's target product profiles (TPP) for new onchocerciasis tests set minimum criteria for mapping as a laboratory-based assay to detect exposure to *Onchocerca volvulus (O.volvulus)* with ≥ 60% sensitivity and ≥ 99.8% specificity. The minimum criteria for tests to make MDA stopping decisions have the same specificity but higher sensitivity (≥ 89%). The requirement for very high specificity for both program use cases may necessitate the use of multiple biomarkers in combination, as studies has shown the necessary tool with a high specificity could not be achieved using a single antigen, such as the *Ov16* based antibody-based detection. Therefore, with that understanding, we sought to identify more potential antigens that could be utilized to this end. We conducted a serum epitope repertoire analysis (SERA) to identify biomarkers to detect anti-IgG, anti-IgG1, and anti-IgG4 epitopes using sera from 60 *O. volvulus* confirmed positive individuals and 60 *O. volvulus* negative individuals from Guatemala and 60 *Wuchereria bancrofti* positive sera from Haiti to control for cross-reactivity. SERA identified 22 target proteins for IgG, 18 for IgG1, and 11 for IgG4. Two identified proteins were previously described antigens, ***OvCol-1*** and ***Ov7***. We expressed these two proteins as GST-fused recombinant antigens and developed a multiplex bead assay (MBA).

OBJECTIVES

- To develop more effective tests with high sensitivity and specificity for monitoring residual infection levels in endemic areas.
- To Identify additional antigens that could be used alone or in combination with *Ov16* for Onchocerciasis MDA screening and stopping.

METHODS

SERA

SERA incorporates a large random peptide library, Next Generation Sequencing (NGS), and custom bioinformatics tools to map immunogenic antigen epitopes targeted for a given infection.
An antibody-containing patient sample (typically serum or plasma) is incubated with a bacterial library displaying random peptides at the cell surface. Antibodies bind to peptide library members that mimic the binding site on their natural protein target. Antibody (IgG, IgG1, and IgG4 subclasses) bound bacteria are magnetically separated to isolate a unique set of antibody-binding peptides for each patient. NGS is used to sequence each patient's set of millions of antibody-binding peptides (i.e. each patient's antibody epitope repertoires). From these peptide datasets, custom computer algorithms identify amino acid motifs that occur in the diseased patient samples and are absent from controls. Motifs are aligned to proteome sequences of interest to identify likely antigen candidates eliciting the patient's antibody response. The most sensitive and specific motifs and antigens are identified for the disease cohort compared to controls and additional databased samples. A validation cohort of samples is then used to evaluate the performance of the selected motifs and antigens. The best-performing antigens are candidates for testing using a simple diagnostic platform such as ELISA or lateral flow (Kamath, K, et al. Sci Rep. 10, 5294 (2020); Reifert, J, et al. J Clin Microbiol. 59, e01836-20 (2021); Pantazes, RJ, et al. Sci Rep. 6, 30312 (2016); Haynes, WA, Kamath, K, Wait,z R, Daugherty, PS, Shon, JC. Front Immunol. 12, 625311 (2021)).

Recombinant Protein Expression and Purification

The proteins were expressed using *E. coli* BL21 DE3 bacterial expression system and purified using the glutathione *S-transferase* (GST) column. Western blot was done on the purified proteins to identify the targets based on size and specificity (Tsang et.al.,1989).

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RESULTS

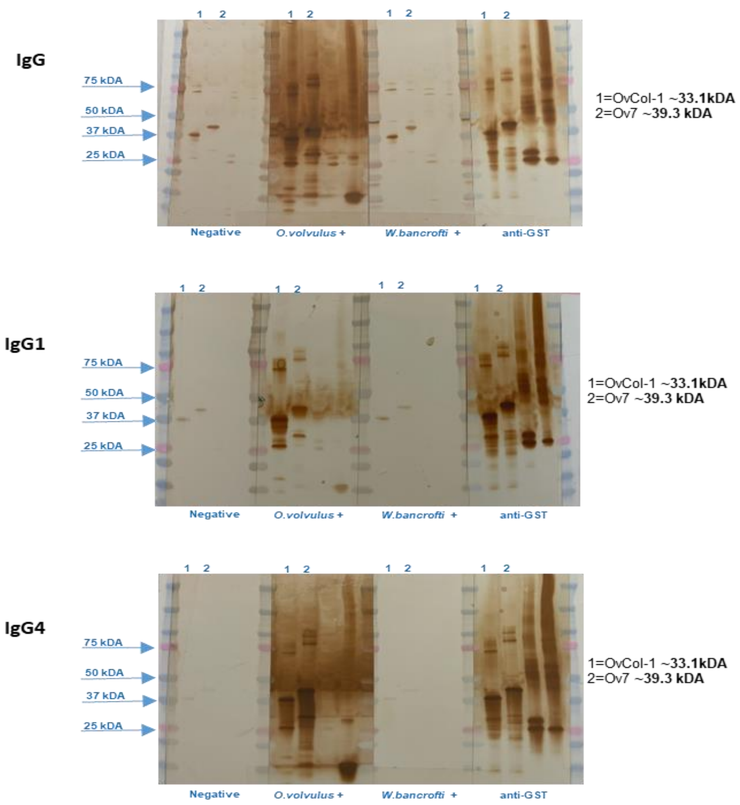

Figure 1: Western blot of the OvCol-1 and Ov7 proteins run against negative sera, *O.volvulus* positive and *W. bancrofti* positive sera using IgG, IgG1- and IgG4- specific antibodies.

Multiplex Bead Assay

The proteins were coupled to magpix beads (microspheres) in an MBA format. Both antigens showed high signal to noise (S/N) ratios against the antibodies tested(~1200 - 34000) obtained using the median fluorescent intensity (MFI) from the assay run on Magpix assay analyzer (**Table 1**). To identify the best coupling buffer, the average MFI was used to calculate the S/N) ratio. Briefly, the result from the "signal" serum (the MFI from the ***O. volvulus*** positive serum) was divided by MFI of the "noise" serum (the MFI of the negative ("normal" serum) or *W. bancrofti*). A S/N ratio greater than 1 shows that more reactivity was seen with the *O. volvulus* serum than the comparator serum sample. The higher the S/N ratio, the greater the difference is between the reactivities of the 2 sera.

Table 1: Optimized coupling of the proteins to Magpix beads for MBA showing the S/N ratio of the positive control (*O. volvulus*) against negative control and *W. bancrofti* (cross reactor)

	OvCol_1			Ov7		
	IgG	*IgG1*	*IgG4*	*IgG*	*IgG1*	*IgG4*
Negative	25	1	1	27	19	3
O.volvulus (Ov)+	32189	34206	10992	33388	36043	45063
W.bancrofti (Wb)+	29	1	1	48	2	5
S/N Negative	1305	34206	10992	1252	1931	15021
S/N Wb	1123	34206	10992	700	18021	9656
Buffer	Borate, pH 8.0	PBS, pH 7.2	PBS, pH 7.2	MES, pH 5.0	PBS, pH 7.2	MES, pH 5.0
Protein amount (ug/ 1X coupling scale)	1.0	1.0	3.0	3.0	6.3	3.0

All values in median fluorescent intensity (MFI)

After protein amount and buffer was optimized, assay linearity was determined using a standard curve. Inter-plate **(Table 2)** and Intra-plate (data not shown) variation were determined from testing the linearized positive and negative sample 24 times. Data were tabulated and inter-plate coefficient variation was calculated using Microsoft Excel.

RESULTS (CONT'D)

Table 2: Inter-plate variation of the proteins showing the coefficient of variation from running the linearized positive and negative sample 24 times on different days in consideration of assay variability and reproducibility.

IgG	Ovcol-1		Ov7	
	Neg	Pos	Neg	Pos
Mean	11.2	7463.4	15.4	8556.4
SD	2.8	607.7	3.3	1220.5
CV	24.9	8.1	21.5	14.3
Mean - 2SD	5.7	6248.1	8.8	6115.3
Mean + 2 SD	16.8	8678.7	22.0	10997.4
IgG1	**Ovcol-1**		**Ov7**	
	Neg	Pos	Neg	Pos
Mean	8	8102	8	7099
SD	6	1156	2	1243
CV	76	14	30	18
Mean - 2SD	-4	5791	3	4614
Mean + 2 SD	21	10413	13	9584
IgG4	**Ovcol-1**		**Ov7**	
	Neg	Pos	Neg	Pos
Mean	2	1738	1	19294
SD	2	316	1	1563
CV	84	18	40	8
Mean - 2SD	-1	1107	0	16167
Mean + 2 SD	6	2370	2	22420

ROC curve graphical representation showing the sensitivities and specificities of the both proteins for IgG. IgG1 and IgG4 reactivity. Determination of the cut-off value and assay performance (sensitivity and specificity) was obtained using R statistical software and pROC package. To combine the results of diagnostic tests, we used a method which finds an optimal linear combination of MFI values of multiple antigens. In this study, a total of 494 *O. volvulus* positive, 64 *W. bancrofti* positive, 197 normal human serum (negative sera) were tested.

Table 3: Performance of *O. volvulus* in MBA

	OvCol-1			Ov7			Combo
	IgG	IgG1	IgG4	IgG	IgG1	IgG4	IgG4*
Threshold	58.6	116.5	5.5	62.5	7.5	2.5	0.241
Sensitivity	90.4	85.6	70.7	83	90.4	93.4	93.9
Specificity	90.5	95.4	97.9	91.2	78.9	95.4	95.4

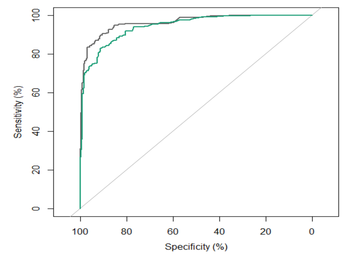

Figure 3A: ROC curve of both proteins against IgG

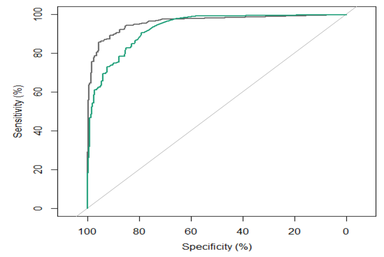

Figure 3B: ROC curve of both proteins against IgG1

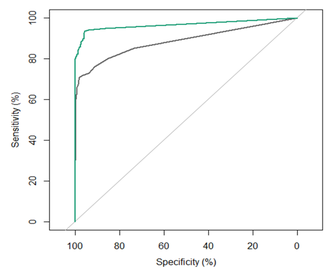

Figure 3C: ROC curve of both proteins against IgG4

DISCUSSION and CONCLUSION

Antigens that can be used for immunodiagnosis of onchocerciasis could benefit both control programs (at elimination mapping and MDA stopping stages) as well as individual patient (travelers and immigrants) diagnosis. In this study, using SERA, potential candidates were identified and evaluated by both immunoblot and MBA. The proteins in this study (***OvCol-1 and Ov7***) were highly specific for *O. volvulus* and showed the littlest cross reactivity with the negative control or *W. bancrofti* positive sera in either IgG4-specific Western Blot or MBA. These proteins demonstrated very strong (~1200 - 34000) signal to noise ratio (S/N) compared to potentially cross-reactive sera from persons with *W. bancrofti* infection in multiplex assays. Two defined sets of *O. volvulus*-positive sera, from different geographical regions were used to optimize and validate the antigens. As individual antigens, IgG4 reactivity of sera from *O.volvulus* infected persons was higher with ***OvCol-1*** having a specificity of 97.9% compared to the ***Ov7*** of 95.4% specificity. ***Ov7*** produced a more sensitive test than ***OvCol-1*** across the all the 3 antibodies tested. By themselves, none of these proteins met the WHO criteria of target product profile (TPP) for onchocerciasis stopping determination (specificity ≥98.9%, and sensitivity ≥89%). However, the combination of both proteins only increased the sensitivity to 93.9% but not specificity when detecting IgG4. It may be possible to use these two antigen to develop an assay for onchocerciasis MDA stopping decisions in combination with Ov16 or other *Onchocerca* antigens, more tests is being done to this effect.

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Biomarker Discovery and Assay Development to Detect Antibody to *Schistosoma haematobium*

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[1] Synergy America Inc; Duluth GA, USA; [2] Centers for Disease Control and Prevention (CDC); Atlanta, GA, USA; [3] **Serimmune; Isla Vista, CA; * = equal contribution**

BACKGROUND

The World Health Organization (WHO) neglected tropical diseases (NTD) road map 2021-2030 highlights the lack of diagnostics to support the control and elimination of schistosomiasis. There are diagnostic needs to decide when to stop mass drug administration (MDA) and subsequent surveillance from detecting the recrudescence of schistosomiasis. The WHO target product profile for this use case is a two-step strategy based on two tests (a combination of point-of-care and laboratory-based or two point-of-care tests). The first screening test should provide higher sensitivity and lower specificity, while the confirmation test provides higher specificity with a lower sensitivity. A test requires multiple antigens to achieve high specificity. Besides that, for areas of co-endemicity, a multiplex assay specific to each species is needed. While several antigens for *S. mansoni* are available, few antigens for *S. haematobium.* Based on this situation, we put effort into finding antibody biomarkers for *S haematobium.*

METHODS

1. Serum Epitope Repertoire Analysis (SERA)

An antibody-containing patient sample (typically serum or plasma) is incubated with a bacterial library displaying random peptides at the cell surface. Antibodies bind to peptide library members that mimic the binding site on their natural protein target. Antibody (IgG total, IgG1, and IgG4 subclasses) bound bacteria are magnetically separated to isolate a unique set of antibody-binding peptides for each patient. Next-Generation Sequencing is used to sequence each patient's set of millions of antibody-binding peptides (i.e. each patient's antibody epitope repertoires). From these peptide datasets, custom computer algorithms identify amino acid motifs that occur in the diseased patient samples and are absent from controls. Motifs are aligned to proteome sequences of interest to identify likely antigen candidates eliciting the patient's antibody response. The most sensitive and specific motifs and antigens are identified for the disease cohort compared to controls and additional databased samples. A validation cohort of samples is then used to evaluate the 5 60) were subjected to the SERA platform.

2. Peptide Array

The protein sequences of *S. haematobium* from the SERA platform and from secretomes and glycosyl-phosphatidyl inositol (GPI)-anchored proteins were converted into an *S. haematobium* peptide microarray. The resulting arrays contained 1,376 different peptides printed in duplicate and were framed by additional HA (YPYDVPDYAG, 108 spots) and polio (KEVPALTAVETGAT, 108 spots) control peptides. The slides were washed using PBS, pH 7.4 with 0.05% Tween 20 for 3 x 10 sec followed by a 30-minutes blocking step in Rockland blocking buffer MB-070. The slides were then exposed to *S. haematobium* positive, negative, and *S. mansoni* positive sera diluted 1:200 (IgG total and IgG4) and 1:100 (IgG1) in 10% blocking buffer. Sera incubation was carried out for 16 h at 4°C and orbital shaking at 140 rpm. After washing as above, goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml), mouse anti-human IgG4 DyLight800 (0.2 µg/ml), and mouse anti-human IgG1 Cy3 (0.2 µg/ml) were added for 45 minutes staining in the blocking buffer at RT. The fluorescence was read by a scanner (LI-COR Odyssey Imaging System) with scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm/green = 800 nm) and Genepix Imaging System, resolution 10 µm, gain of 500 PMT(red = 632 nm / green = 532 nm)

3. Peptide ELISA

Peptides identified through the SERA platform and peptides that showed significant signal-to-noise ratios in the peptide array were synthesized using the service of a commercial company. After peptide resuspension to the respective diluent at 2 mg/mL, peptides were diluted to 10 ug/mL in sensitization buffer (50 mM Tris-HCl, ph 8.0 + 0.3 M KCl + 2 mM EDTA) and added to wells and then peptide sensitization was carried out at 4 C overnight.

The plate was washed with PBS/0.3% Tween-20 and then diluted serum (1:50 in PBS/0.3% Tween-20/5% milk) was added to the wells. After 30 minutes of incubation, the plate was washed and secondary antibodies (either IgG total, IgG1, or IgG4) – HRP conjugated was added. After a further 30 minutes of incubation, the plate was washed, and TMB substrate was added. The reaction was observed at A650 nm.

4. Protein Expression

Gene sequence of precursor anti-coagulant SAP-1 *S. haematobium* (AAD0056.1) was synthesized, subcloned into pGS21a expression vector, transformed into BL21(DE3) *E. coli,* and expressed as fusion protein after IPTG induced treatment. The specific coupled magnetic beads were used to capture and purify the GST-HIS tagged protein.

5. Magnetic Bead Assay

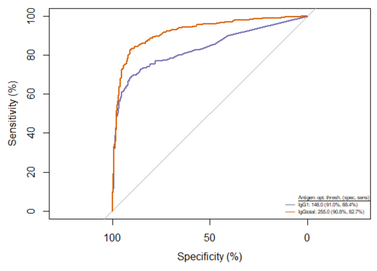
rGST-SAP1 was coupled into magnetic beads and was qualified using a set of defined eggs positive *S. haematobium sera,* negative sera, and cross-reactors. The sensitivity and specificity of the assay were determined using a cut-off point from the ROC curves for IgG total and IgG1 antibody detection

RESULTS

Peptide #	Motif	Candidate Epitope	Putative Antigen	Subclass	IgGT		IgG1		IgG4	
					Sh	Sm	Sh	Sm	Sh	Sm
1	VSxRPxxK	KE**VSKRPVQK**HV	Precursor anti-coagulant SAP-1	IgGT / IgG1	62%	8%	30%	0%	0%	0%
2	PAx[AP]N[IVC]	QGK**PSPPNI**TSV	Fibronectin type 3 and ankyrin repeat domains protein 1	IgGT / IgG1	58%	2%	30%	3%	0%	2%
3	[IVLP]DEYP	QNDV**VDEYP**E	200-kDa GPI-anchored surface glycoprotein	IgGT / IgG1	58%	0%	27%	0%	2%	0%
4	PYxVVxH	IK**PYPVVVY**NR	Putative heparan sulfate 2-o-sulfotransferase	IgGT / IgG1	45%	0%	22%	3%	0%	0%
5	PYPVx[FY]	DDV**PYPVTY**HM	N/A (Peptide binder)	IgGT / IgG1	45%	0%	20%	2%	3%	0%
6	HDTMG	AVM**HDTM**DV	Voltage-dependent calcium channel	IgGT / IgG1	37%	0%	10%	5%	7%	2%
7	TDExx[LI]F	LKPP**TDEEQLF**K	Uncharacterized protein	IgGT / IgG4	55%	3%	7%	2%	45%	0%
8	Cxx[FL]GCF	DNMV**CYTFGCF**G	N/A (Peptide binder)	IgGT / IgG4	17%	2%	0%	5%	40%	5%
9	[TS]VDxWM	YRY**SVDEWM**FVH	N/A (Peptide binder)	IgGT / IgG4	28%	0%	0%	0%	35%	0%
10	D[ND]H[ILM]W	DMV**DDHLW**SKA	Putative mbp-1	IgGT / IgG4	30%	2%	10%	2%	15%	0%

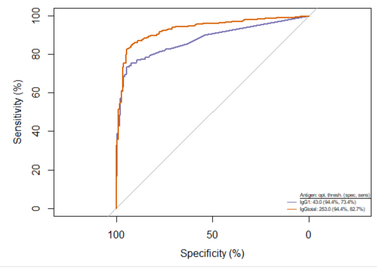
The potential as antigen of peptides of precursor anti-coagulant SAP-1 to detect antibody (IgG total and IgG1) in *S. haematobium* infected subjects was confirmed in peptide array and peptide ELISA. We followed by expression and purification of the whole sequence of this SAP-1 protein and used it for a magnetic bead assay.

ROC curve using all sera *

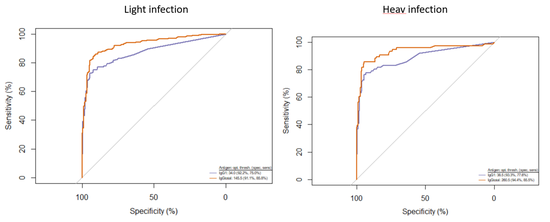


Negatives = US negative + cross reactors (*S. mansoni-S japonicum-Malaria-Cysticercosis-Strongyloidiasi.*

ROC curve using US negatives



ROC curves based on infection intensity



rSh-SAP-1 MBA performance *

Criteria	IgG1	IgG total
Cut-off point	43	253
Sensitivity	73	83
Specificity	94	94

Note: * = based on US negative ROC curve

Cross-reactivities to several conditions

Condition	% reactivities (n/N) to IgG1 Cut-off = 43 *	% reactivities (n/N) to IgG total Cut-off = 253 *
US negative	5.4 (9/168)	6.0 (10/168)
Cysticercosis	40.6 (13/32)	0 (0/32)
Malaria	26.8 (11/42)	0 (0/42)
Schistosomiasis japonicum	25.7 (9/35)	14.3 (5/35)
Schistosomiasis mansoni		
- Origin: Sub-Saharan countries	30.6 (22/72)	27.8 (20/72)
- Puerto Rico	23.0 (3/13)	30.8 (4/13)
Strongyloidiasis	6.2 (5/81)	3.7 (3/81)

Note: * = based on US negative ROC curve

DISCUSSION AND CONC

SERA platform identified several potential peptides for the detection of antibodies (IgG total and IgG1) in subjects with *S. haematobium* infection. One peptide mapped to SAP-1 *S. haematobium* showed similar reactivities in peptide array and peptide ELISA and successfully developed into a magnetic bead assay. This antibody marker was qualified. Cross-reactivity to *S. mansoni* was predicted using SERA.

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