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

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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

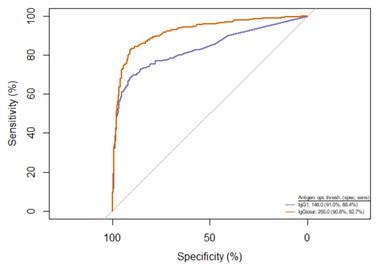
rGST-SAP1 was coupled into magnetic beads and was qualified using a set of defined eggs positive *S. haematobium sera,* negative sera, and cross-reactors. The sensitivity and specificity of the assay were determined using a cut-off point from the ROC curves for IgG total and IgG1 antibody detection

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%

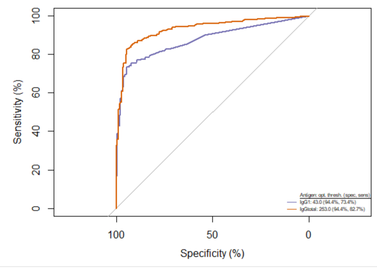
The potential as antigen of peptides of precursor anti-coagulant SAP-1 to detect antibody (IgG total and IgG1) in *S. haematobium* infected subjects was confirmed in peptide array and peptide ELISA. We followed by expression and purification of the whole sequence of this SAP-1 protein and used it for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

ROC curve using all sera *

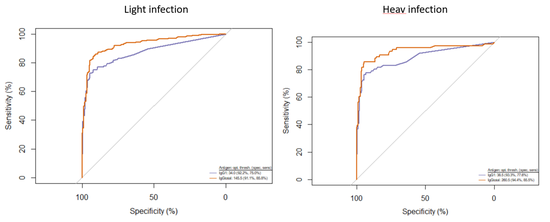


Negatives = US negative + cross reactors (*S. mansoni-S japonicum-Malaria-Cysticercosis-Strongyloidiasi.*

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.

SCAN HERE FOR MORE INFORMATION

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.