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Original Article

Enhancing serological diagnosis of urogenital schistosomiasis with admixtures of antigens from *Schistosoma* eggs and worms

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Abstract

Introduction: Timely and effective diagnosis plays a pivotal role in schistosomiasis control efforts. This study aims to assess the utility of combined *Schistosoma haematobium* soluble egg antigen (*Sh* SEA) and *S. mansoni* worm antigen (*Sm* SWA) in serological diagnosis of urogenital schistosomiasis.

Methodology: Admixtures containing 10 µg/mL of both *Sm* SEA and *Sh* SEA, as well as *Sm* SWA and *Sh* SWA, were employed to detect *S. haematobium* infection via an indirect enzyme-linked immunosorbent assay (ELISA) using sera and urine from microscopically confirmed positive samples from an endemic population, along with confirmed negative samples from both endemic (NE) and non-endemic (NNE) populations.

Results: The diagnostic performance of *Schistosoma* eggs and worm antigen mixtures varied depending on sample type and negative endemicity. The *Sm* SEA and *Sh* SEA mixtures performed poorly with sera and urine from the pair of positive *vs* negative endemic samples, as well as positive *vs* non-endemic samples, but excellently with positive *vs* negative endemic urine samples pair (sensitivity 91.67%; specificity 66.67%). Conversely, SWA mixtures showed superior performance, particularly with the positive *vs* negative non-endemic sera samples pair (sensitivity 93.75%; specificity 72.92%). Other SWA-based mixtures, except SWA admixture using urine in positive *vs* NE samples, exhibited acceptable performance. Antibody titers varied significantly, with higher titers generally observed in negative endemic samples for SWA mixtures and in negative non-endemic urine samples for SEA mixtures ($p < 0.05$).

Conclusions: Combined antigens improve *Schistosoma* diagnostics: SEA admixtures suit endemic urine samples, while SWA admixtures aid non-endemic sera detection.

Key words: *Schistosoma* antigen admixtures; diagnostic performance; schistosomiasis control; endemicity level.

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Introduction

Schistosomiasis transmission persists without abatement across various regions globally, with a notable concentration in sub-Saharan Africa [1]. Comprehensive strategies encompass treatment, disrupting transmission via snail control, swift and effective diagnosis, and mitigating human-water contact by ensuring access to safe water and promoting proper hygiene and sanitation facilities. Diagnosis plays a central role in achieving the 2030 goal of eliminating schistosomiasis, serving as a critical element in neglected tropical disease (NTD) surveillance and evaluating intervention effectiveness [2].

Microscopy has emerged as the primary diagnostic method in numerous endemic regions, yet its efficacy is often hindered by laboriousness and inaccuracies, especially in scenarios with low parasite density [3]. In cases of chronic infection, microscopy may prove inadequate in detecting true infection due to disease-related complications that facilitate the retention of parasite eggs within the human host. In resource-limited settings endemic to urogenital schistosomiasis, the

visible presence of blood or the microscopic identification of blood in urine, along with proteinuria using chemical reagent strips, have become valuable tools for delineating areas with urogenital schistosomiasis prior to treatment [4]. However, this method may not be suitable for non-endemic areas or populations with low *Schistosoma* intensity [5].

Serological diagnostic techniques have been extensively investigated to offer a more sensitive approach for diagnosing schistosomiasis, particularly in settings with low transmission rates [6,7]. Notably, the utilization of *Schistosoma* antigens derived from various stages of the trematode as antibody capture agents in immunoassays has garnered interest. *Schistosoma* egg antigen (SEA) and worm antigen (SWA) have been widely utilized and have demonstrated impressive diagnostic efficacy in specific instances, exhibiting sensitivities ranging from 36 to 96% [8].

While point-of-care (POC) diagnostic devices have revolutionized infectious disease diagnosis, advancements in schistosomiasis diagnosis have been comparatively limited. Although a POC device suitable for detecting circulating cathodic antigen (CCA) of *S. mansoni* in patient urine is commercially available, a corresponding tool for *S. haematobium* is still lacking in the market. POC-CCA faces similar challenges of inadequate infection detection in regions with low transmission rates [9], underscoring the necessity for further investment in schistosomiasis diagnostic development.

Considering the inconsistent diagnostic performance of SEA and SWA in certain cases, coupled with the presence of shared proteins in both *S. mansoni* and *S. haematobium* [10], combining antigens from both trematodes could enhance diagnostic accuracy. This approach aligns with the principle of utilizing multivalent vaccines to enhance immunogenicity [11]. The objective of the current study is to assess the diagnostic effectiveness of combining SEA from *S. mansoni* and *S. haematobium*, as well as the combination of SWA from both trematodes.

Methodology

Crude antigen preparations

The *Schistosoma mansoni* and *S. haematobium* antigens (SEA and SWA) were sourced from the Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute, Giza, Egypt. The antigens' preparations are briefly described as follows: *Schistosoma* cercariae were harvested from infected snails reared in the Schistosome Biological Supply Unit at the Theodor Bilharz Research Institute (Giza, Egypt) and maintained in the laboratory following the protocols outlined by Becker and Lamprecht [12]. After light exposure for at least 4 hours, the cercariae were used to infect mice (*S. mansoni*) or hamsters (*S. haematobium*) via subcutaneous injection. All animal procedures adhered to the International Guiding Principles for Biomedical Research, as established by the International Organizations of Medical Science [13].

Eggs were isolated from liver tissues and suspended in cold PBS (4°C) at a concentration of 100,000 eggs/mL. The eggs were homogenized on ice using a pre-chilled homogenizer with a tight pestle. The resulting crude mixture was centrifuged at 4°C (200 × g, 20 minutes), and the supernatant was collected and further centrifuged (100,000 × g, 90 minutes, 4°C). The clarified supernatant was sterilized by filtration through a 0.2-µm pore membrane [14]. Similar procedures were performed for the worm antigens. Finally, the protein concentration of the prepared antigens was quantified using Bradford's method [15].

Samples source

Fifty (50) sera samples stored at -20˚C in the sample repository of the Seeding Graduate Laboratory at the University of Medical Sciences, Ondo, Nigeria, were used for the analysis. These samples were sourced from an endemic region of Katsina State, Northern Nigeria, gathered between April and May 2022. Parasitological methods, including microscopy to demonstrate eggs in stool samples and urine sedimentation by centrifugation, were utilized to classify the samples into confirmed positive and negative categories based on schistosomiasis infection. Samples showing co-infections with other parasites were excluded based on the results of the parasitological analysis. Additionally, 50 sera collected from students and staff at the University of Medical Sciences, Ondo, who lacked a history of human-water contact with any river were confirmed as negative non-endemic samples via microscopy. These samples underwent screening to detect the presence of schistosomiasis and other parasitic infections.

Indirect-ELISA procedure

The selected samples underwent screening for IgG antibodies using an indirect ELISA procedure. The indirect ELISA protocol followed the methodology outlined by Oyeyemi *et al.* [7] with minor adjustments. Each well of the microtiter plate (APS LIFETECH WEST AFRICA) was incubated overnight at 4 °C with

100 µL of a mixture containing 10 µg/mL of *Sm* SEA and *Sh* SEA (50 µL each of *Sm* SEA and *Sh* SEA; each at a concentration of 5 µg/mL), diluted in coating buffer (carbonate-bicarbonate). Subsequently, the plate underwent five washes with phosphate buffer saline (PBS) (1×) containing 0.05% Tween 20 (PBST-washing buffer). The microtiter plate was then blocked with 300 µL per well of 2.5% skim milk diluted in washing buffer, followed by a 2-hour incubation at 25 °C. After another five washes, each well received 100 µL of serum/urine sample (diluted 1:100 in PBS (1×)) and was incubated at 25 °C for 1 hour. Following a further five washes, the plate was incubated for 1 hour at 25 °C with anti-human-IgG horseradish peroxidase-conjugated antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:10,000 in washing buffer. After five additional washes, 100 µL of substrate solution (3'3′,5'5-tetramethylbenzidine) (Elabscience Biotechnology Co., Ltd) was added to each well. The reaction was halted after a 10-minute incubation period by adding 100 µL of 2N sulfuric acid per well. All assays were conducted in duplicate, and the absorbance OD was read at 450nm using a microplate reader (BIOBASE BK-EL10C). The same procedures were repeated for a mixture of *Sm* SWA and *Sh* SWA.

Statistical analysis

Mean optical density (OD) values obtained from the microplate reader were analyzed using GraphPad Prism® version 8.0.1. To compare antibody levels between positive and negative individuals, a Student's *t*-test was employed. Results were reported as mean ± SD and along with various diagnostic metrics, including the area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, and specificity. The AUC served as an indicator of diagnostic performance, where values of 0.5 suggested no discrimination, 0.7-0.8 were considered acceptable, 0.8-0.9 indicated excellent performance, and values above 0.9 suggested outstanding performance [16]. Statistical significance was defined as $p < 0.05$. The selected cut-off OD from GraphPad Prism was used to classify sera samples into positive and negative groups (both negative endemic and negative non-endemic). Diagnostic accuracy, defined as the proportion of correct test results, was calculated using the formula: (number of true positives + number of true negatives) / (number of true positives + number of true negatives + number of false positives + number of false negatives).

Results

The diagnostic performance of crude *Schistosoma mansoni* (*Sm*) and *S. haematobium* (*Sh*) antigen admixtures was assessed using sera and urine samples, with comparisons against negative endemic (NE) and non-endemic (NNE) samples. In sera, the *Sm* SEA + *Sh* SEA combination exhibited lower true positives (TP = 19, positive *vs* NE) and higher false positives (FP = 36, positive *vs* NNE), while *Sm* SWA + *Sh* SWA showed improved detection (TP = 40, positive *vs* NE). For urine samples, *Sm* SEA + *Sh* SEA demonstrated high true positives (TP = 44 *vs* NE; 38 *vs* NNE), whereas *Sm* SWA + *Sh* SWA achieved even better performance (TP = 39 *vs* NNE) with minimal false negatives (FN = 9) (Table 1).

The diagnostic performance of *Sm* and *Sh* antigen admixtures varied significantly by sample type (sera/urine) and endemicity status (NE/NNE). SEA admixtures demonstrated markedly higher sensitivity in urine samples from positive *vs* endemic negative endemic samples (91.67%, 95% CI: 80.45–96.71; AUC = 0.8542, $p < 0.0001$), though specificity remained moderate (66.67%, 95% CI: 52.54–78.32). In contrast, SEA admixtures performed poorly in sera, with AUC values near chance (0.5130–0.5608) and low specificity (25.00–50.00%) ($p > 0.05$).

SWA admixtures exhibited superior performance in sera, especially for NNE samples (sensitivity: 93.75%, 95% CI: 83.16–97.85; specificity: 72.92%, 95% CI: 59.00–83.43; AUC = 0.9193, $p < 0.0001$). However, SWA admixtures in urine showed limited utility for positive *vs* NE samples (AUC = 0.5012, sensitivity: 28.83%), while positive *vs* NNE urine samples achieved acceptable discrimination (AUC = 0.7135, $p = 0.0003$). Notably, the laboratory reference tests using single antigens (*Sh* SEA or *Sh* SWA) showed comparable sensitivity (64.7–92%) but variable specificity (50–81.3%), underscoring the improved

Table 1. True and false negative/positive diagnostic values of crude antigen admixture using sera and urine samples.

Sample Antigens	Sera				Urine			
	Sm SEA + Sh SEA		Sm SWA + Sh SWA		Sm SEA + Sh SEA		Sm SWA + Sh SWA	
	Positive vs NE	Positive vs NNE	Positive vs NE	Positive vs NNE	Positive vs NE	Positive vs NNE	Positive vs NE	Positive vs NNE
TP	19	38	40	45	44	38	10	39
FP	24	36	20	13	16	32	24	19
TN	24	12	28	35	32	16	24	29
FN	29	10	8	3	4	10	38	9

Table 2. Diagnostic potential of admixture of SEA and SWA.

Antigen admixture	Sample	Positive vs non-infected samples	AUC	Cut-off	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	*p*
Sm SEA + Sh SEA	Sera	Positive vs NE	0.5130	> 0.9530	39.58 (25.77-54.73)	50.00 (36.39-63.61)	0.8260
Sm SEA + Sh SEA	Sera	Positive vs NNE	0.5608	> 0.7972	79.17 (65.74-88.27)	25.00 (14.92-38.78)	0.3050
Sm SEA + Sh SEA	Urine	Positive vs NE	0.8542	> 0.4732	91.67 (80.45-96.71)	66.67 (52.54-78.32)	< 0.0001
Sm SEA + Sh SEA	Urine	Positive vs NNE	0.5827	> 0.6667	79.17 (65.01-89.53)	33.33 (21.68-47.46)	0.1627
Sm SWA + Sh SWA	Sera	Positive vs NE	0.7088	> 0.9446	83.33 (70.42-91.30)	58.33 (44.28-71.15)	0.0004
Sm SWA + Sh SWA	Sera	Positive vs NNE	0.9193	> 0.7880	93.75 (83.16-97.85)	72.92 (59.00-83.43)	< 0.0001
Sm SWA + Sh SWA	Urine	Positive vs NE	0.5012	< 0.4557	28.83 (10.74-34.99)	50.00 (36.39-63.61)	< 0.0001
Sm SWA + Sh SWA	Urine	Positive vs NNE	0.7135	> 0.3151	81.25 (68.06-89.81)	60.42 (46.31-72.98)	0.0003

NE – *Sh* SEA: *Schistosoma haematobium* soluble egg antigen; *Sm* SEA: *Schistosoma mansoni* soluble egg antigen; *Sh* SWA: *Schistosoma haematobium* worm antigen; *Sm* SWA: *Schistosoma mansoni* worm antigen negative endemic; NNE: negative non-endemic. Laboratory reference using sera – *Sh* SEA; sensitivity 90%, specificity 50/72% (NE/NNE); *Sh* SWA: sensitivity 64.7/92% (NE/NNE), specificity 81.3/72% (NE/NNE).

balance of sensitivity and specificity achieved by SWA admixtures in NNE sera (Table 2).

Figures 1 and 2 display the ROC curves representing the performance of *Sm* SEA/*Sh* SEA and *Sm* SWA/*Sh* SWA mixtures in ELISA results of screened samples, employing positive *vs* negative endemic and positive *vs* non-endemic sera and urine samples. Specifically, in Figure 1C, the ROC curves of *Sm* SEA and *Sh* SEA mixtures using urine and positive *vs* negative endemic samples, as well as in Figure 2B, *Sm* SWA and *Sh* SWA using sera and positive *vs* negative non-endemic samples, and in Figure 2C, *Sm* SWA and *Sh* SWA using urine and positive *vs* negative endemic samples, all leaned towards the upper left-hand corner in the ROC space. However, in Figures 1A and 1B, the ROC curves of *Sm* SEA and *Sh* SEA mixtures using sera and positive *vs* NE samples, as well as using sera and positive *vs* NNE samples, respectively, appeared to somewhat align along the diagonal line.

Figure 3 displays the mean anti-*Schistosoma* IgG levels generated against the diagnostic antigen mixtures, as measured by optical density (OD). For *Sm* SEA and *Sh* SEA mixtures, the mean OD values using sera from both negative endemic and negative non-endemic samples showed no significant differences between positive and negative samples ($p > 0.05$). However, for the same mixtures using urine, a significantly higher antibody titer was observed in NNE samples (mean OD: 0.71 ± 0.07) compared to NE samples (mean OD: 0.50 ± 0.2) ($p < 0.05$). On the other hand, the mean antibody titer against *Sm* SWA and *Sh* SWA mixtures was significantly higher when sera from

Figure 1. Receiver operating characteristics (ROC) curve of diagnostic performance of SEA admixtures. **A.** Sh-SEA + Sm SEA, Sera NE; **B.** Sh SEA + Sm SEA, Sera NNE; **C.** Sh SEA + Sm SEA, Urine NE; **D.** Sh SEA + Sm SEA, Urine NE.

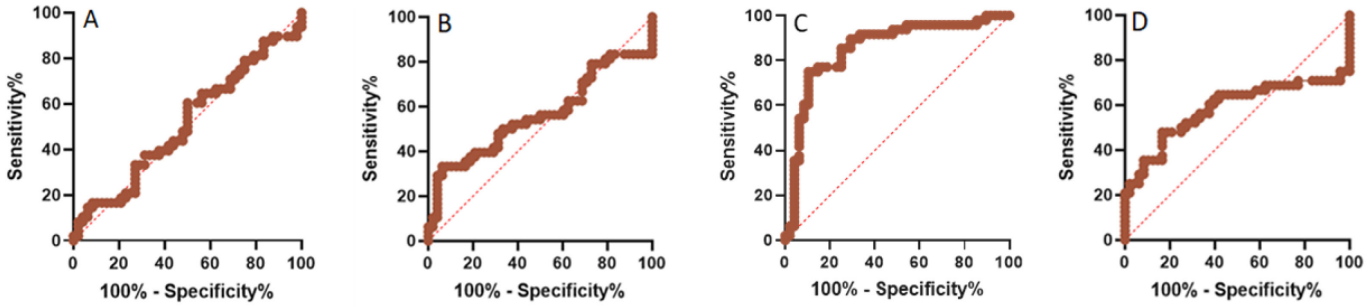
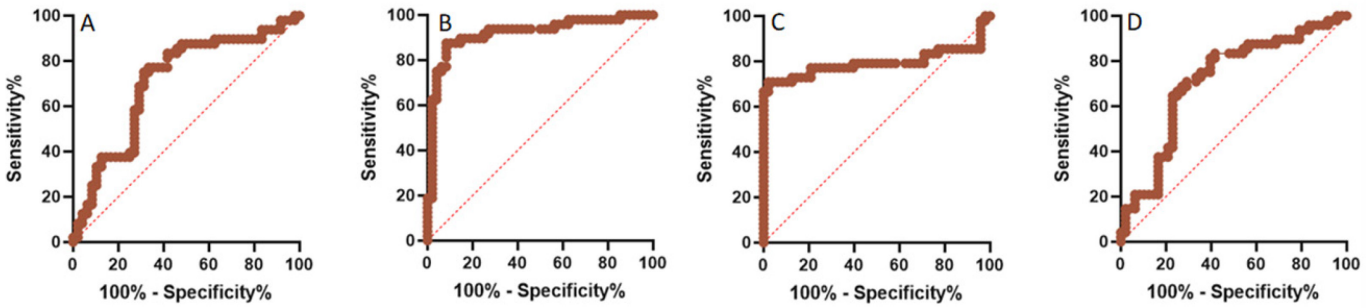


Figure 2. Receiver operating characteristics (ROC) curve of diagnostic performance of SWA admixtures. **A.** Sh-SWA + Sm SWA, Sera NE; **B.** Sh SWA + Sm SWA, Sera NNE; **C.** Sh SWA + Sm SWA, Urine NE; **D.** Sh SWA + Sm SWA, Urine NE.



NE samples (mean OD: 0.96 ± 0.19) were utilized compared to NNE samples (mean OD: 0.74 ± 0.14) ($p < 0.05$). Similarly, a significantly higher antibody titer was observed in SWA mixtures probed against urine from NE samples (mean OD: 0.49 ± 0.12) compared to NNE samples (mean OD: 0.34±0.11) ($p < 0.05$) (Figure 3).

Discussion

The importance of diagnosis in achieving the 2030 elimination target of schistosomiasis has been underscored by researchers [2]. It serves as a pivotal tool in monitoring the effectiveness of mass drug administration and identifying transmission in emerging areas. Moreover, in cases of prolonged chronic conditions where *Schistosoma* parasites are present but not excreted in eggs, traditional microscopy may fail to detect low-intensity infections. Given these complexities, there is a pressing need for diagnostic methods that can accurately determine the true infection status. Efficient diagnostic approaches are essential to address these challenges and ensure effective disease management.

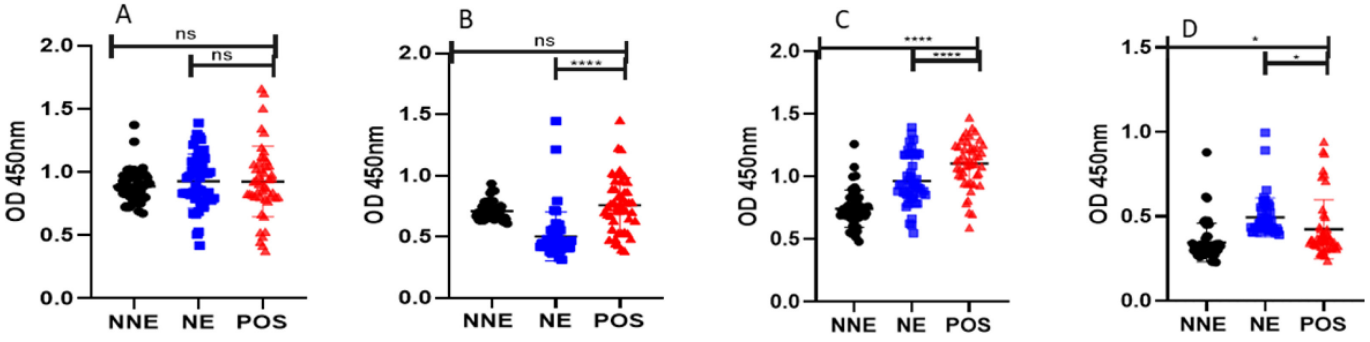
Studies have extensively reported serological-based diagnostics utilizing *S. mansoni* antigens, while fewer have focused on *S. haematobium* [6,7,17]. Our diagnostic methodology employs the mixing of SEA, particularly suited for urine samples in endemic regions. Remarkably, our SEA admixture exhibited a sensitivity of 91.7%, surpassing the 90% sensitivity of our laboratory's reference using sera and the 90.7% sensitivity observed in a separate study utilizing *Sh* SEA alone as the capture antigen [18]. Although our sensitivity fell short of the 95.6% achieved with *Sh* SEA in another investigation [19], our approach demonstrated significantly higher specificity at 66.7% compared to 32%, thereby enhancing the overall diagnostic efficacy of our SEA admixture. This improvement is particularly advantageous as it streamlines sample collection, facilitating the development of a non-invasive diagnostic strategy.

The observed differential performance of *Schistosoma* antigen admixtures, with SEA showing superior detection in urine samples from endemic areas and SWA admixture performing better in sera from non-endemic regions, warrants deeper immunological consideration. In endemic settings, chronic *S. haematobium* infection results in continuous egg deposition in bladder tissues, leading to persistent release of immunogenic SEAs into urine that elicit strong local IgG responses [17,18]. The diagnostic advantage of SEA admixtures in urine may further stem from their enhanced ability to capture immune complexes formed during prolonged exposure, coupled with urine's direct reflection of urogenital pathology compared to serum's broader systemic antibody profile that may include cross-reactive antibodies [8].

Similarly, our study revealed superior diagnostic performance with a SWA admixture (sensitivity 93.8%; specificity 72.9%) using sera and positive *vs* negative non-endemic samples, compared to our laboratory reference (sensitivity 92%; specificity 72%) employing only *Sh* SWA. However, our results exhibited slightly lower sensitivity and specificity (sensitivity 93.8 vs. 100%; specificity 72.9 vs. 76.7%) compared to the findings of Sarhan *et al.* [20]. The diminished performance of our diagnostic approach may stem from differing epidemiological contexts. SWA's superior performance in non-endemic sera in our study likely relates to fundamental differences in antigen composition and host immune responses. Adult worm-derived antigens in SWA preparations contain stage-specific epitopes that demonstrate reduced cross-reactivity compared to egg antigens [10], potentially yielding greater specificity in populations with limited exposure history. The transient nature of worm antigens compared to persistent egg antigens may also make SWA more effective for detecting recent infections in non-endemic travelers [6], while their systemic immunogenicity favours serum-based detection [20].

Several knowledge gaps remain regarding these

Figure 3. Antibody titers in response to SEA and SWA admixtures. **A.** Sm SEA + Sh SEA, Sera; **B.** Sm SEA + Sh SEA, Urine; **C.** Sm SWA + Sh SWA, Sera; **D.** Sm SWA + Sh SWA, Urine.



observed patterns. The origin of urinary antibodies, whether locally produced or serum-derived, remains unclear and could significantly impact test interpretation [17]. Furthermore, the study lacks direct evidence of enhanced epitope binding in admixtures compared to single antigens, and potential cross-reactivity with other helminth infections in endemic areas was not systematically evaluated [8]. These limitations highlight the need for more detailed immunological characterization of antigen-antibody interactions in different biological matrices and epidemiological contexts.

The ROC curves for the SEA admixture using urine and positive *vs* negative endemic samples, the SWA admixture using sera and positive *vs* negative non-endemic samples, and the SWA admixture using urine and negative non-endemic samples all exhibit a pronounced leaning towards the upper left-hand corner of the ROC space. This trend signifies the progressively enhanced discriminant capacity of these admixtures in distinguishing between infected and non-infected individuals. The strong diagnostic performance suggested by these findings is quantitatively supported by AUC values, which ranged from 0.71 (indicating acceptable discrimination) to over 0.90 (demonstrating outstanding diagnostic accuracy). Conversely, the ROC curves for the *Sm* SEA and *Sh* SEA mixtures using sera from positive *vs* negative endemic and positive *vs* non-endemic samples align closely along the diagonal line. This alignment suggests the performance of diagnostic tests is no better than chance, where results yield positive or negative outcomes unrelated to the true *S. haematobium* status [21]. These results are further supported by AUC values between 0.5–0.6, indicating poor diagnostic test discrimination between infected and uninfected individuals, alongside the observation that SEA admixtures yielded higher antibody titers in non-endemic negative samples than in endemic samples when tested with sera.

SWA-based admixtures exhibited anticipated patterns of specific antibody responses, where negative endemic sera and urine samples displayed higher antibody titers compared to corresponding negative non-endemic samples. The mean antibody titer values ranged from 0.34 to 0.96, surpassing the range of 0.27 to 0.32 observed when only SWA was employed to assess the anti-*Schistosoma haematobium* response [20]. However, it remains uncertain whether the heightened responses in the admixture are genuinely linked to multiple antigenic epitopes.

Conclusions

Our study highlights the potential enhancement of serological diagnostic capabilities for *Schistosoma* eggs and worm-based antigens through the combination of two antigens. The utilization of an admixture of SEA proves most effective for urine samples collected from confirmed positive *vs* negative endemic regions, which could significantly impact the monitoring of urogenital schistosomiasis in populations with chronic illness. Furthermore, the use of urine suggests the potential applicability of this method for developing non-invasive diagnostics. Conversely, the SWA admixture may be advantageous for utilizing sera in diagnosing urogenital schistosomiasis in non-endemic populations. It is plausible that alternative antigen mixtures could offer superior diagnostic performance compared to the *Schistosoma* antigens examined in our study. Further elucidation of the immune response mechanisms triggered by these admixtures is warranted.

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Research ethics

This study complies with all national regulations, institutional policies, and ethical principles of the Declaration of Helsinki, and was approved by the Research Ethics Committees of the University of Medical Sciences (Approval number: NHREC/TR/UNIMED-HREC-Ondo St/22/06/21) and the Katsina State Ministry of Health (Reference: MOH/ADM/SUB/1152/1/515).

Informed consent

Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Authors' contributions

The authors have accepted responsibility for the entire content of this manuscript and approved its submission. OTO, FIDA, ABO, TA, RFQG – the conception and design of the study. PCI, KAS, TOO, AOA, TA, OTO – data collection. PCI, KAS, TOO, OTO – data analysis and interpretation. RFQG, RGF – materials acquisition. OTO – drafting of the article. FIDA, ABO, RGF, RFQG – revised the article critically for important intellectual content. All authors approved the final draft before submission.

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Data availability statement

The raw data can be obtained on request from the corresponding author.

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Conflict of interest

No conflict of interest is declared.

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