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Utility of recombinant *Schistosoma bovis* 22.6 kDa antigen for diagnosis of human urogenital schistosomiasis by ELISA: a cross-sectional study in Yewa North LGA, Ogun State, Nigeria

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Abstract

Background In many endemic regions, accurately diagnosing urogenital schistosomiasis remains challenging, particularly in cases of low-intensity infection and limited laboratory capacity. Exploring antigens that cross-react between related schistosome species provides a promising alternative to conventional diagnostic approaches. *Schistosoma bovis*—an animal parasite closely related to *S. haematobium* and sharing the same *Bulinus* snail hosts in overlapping habitats— is a strong candidate for such studies. This work therefore assessed whether the recombinant *S. bovis* 22.6 kDa antigen (rSb22.6 kDa) could detect anti-schistosome antibodies in urine samples from individuals living in *S. haematobium*-endemic communities in Nigeria.

Methods Urine samples from 559 individuals (290 adults and 269 children; 235 males and 313 females) from four communities and schools in Yewa North Local Government Area, Ogun State, Nigeria, were collected. Samples were examined for *S. haematobium* eggs by microscopy, screened for anti-schistosomal antibodies using r*Sb* 22.6 kDa ELISA, and analyzed for haematuria, leukocyturia, and proteinuria using reagent strips.

Results Prevalence by microscopy was 13.9% (78/558), while 54.1% (299/553) of samples were seropositive by ELISA. Among ELISA-positive individuals, 29.8% had haematuria, 47.9% had leukocyturia, and 37.1% had proteinuria. Of 480 microscopy-negative individuals, 221 (46.0%) were ELISA-positive. When microscopy was used as the reference standard, the r*Sb* 22.6 kDa-ELISA showed 46.2% sensitivity and 45.8% specificity. However, ELISA positivity correlated significantly with clinical markers of urogenital pathology ($P < 0.05$).

Conclusion The r*Sb* 22.6 kDa antigen demonstrated cross-reactivity with antibodies in urine from individuals in *S. haematobium*-endemic communities. The high seropositivity compared to microscopy prevalence may reflect detection of low-intensity infections, past exposure with persistent antibodies, or ongoing transmission intensity

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in these communities. While the current performance of the assay does not support its use for individual diagnosis, it shows potential for sero-epidemiological surveillance in endemic areas. Further validation studies incorporating molecular diagnostics and longitudinal follow-up will clarify the significance of antibody positivity in the absence of detectable eggs.

Clinical trial number Not applicable.

Keywords Urogenital schistosomiasis, Serodiagnosis, ELISA, Recombinant *S. bovis* 22.6 kDa antigen, Cross-reactivity, Antibody detection

Background

Schistosomiasis remains a major public health challenge in sub-Saharan Africa, with an estimated 200 million people infected and over 700 million at risk [1]. In Nigeria, urogenital schistosomiasis caused by *Schistosoma haematobium* is highly endemic, with approximately 29 million people infected and 101.3 million at risk [2]. Yewa North Local Government Area has been identified as a high-prevalence region where communities depend on river water for domestic, agricultural, and religious purposes, facilitating continuous transmission [3, 4].

Accurate diagnosis is essential for effective schistosomiasis control programs, yet current diagnostic methods have significant limitations. Microscopic detection of *S. haematobium* eggs in urine, the gold standard diagnostic approach, has poor sensitivity in low-intensity infections and requires multiple samples collected over consecutive days to achieve acceptable detection rates [5]. This makes it impractical for large-scale surveillance in resource-limited settings. Serological assays detecting anti-schistosomal antibodies offer advantages of higher sensitivity and simpler sample collection, but most require specialized antigens derived from human schistosomes that are difficult to obtain [6]. Alternative molecular diagnostics such as PCR and loop-mediated isothermal amplification (LAMP) offer improved sensitivity but require sophisticated laboratory infrastructure which is hardly available in many endemic settings. Circulating anodic antigen (CAA) detection shows promise but requires specialized reagents and has variable performance across different epidemiological contexts. Point-of-care circulating cathodic antigen (POC-CCA) tests are field-deployable but show cross-reactivity issues and reduced sensitivity in low-intensity infections.

An emerging concern is the potential for hybridization between *S. haematobium* and the cattle parasite *Schistosoma bovis*, which share overlapping geographical distributions and the same *Bulinus* snail intermediate hosts [7]. Hybrids between these species have been documented in several West African countries including Senegal, Niger, and Côte d'Ivoire [8, 9], and were implicated in an outbreak of urinary schistosomiasis in Corsica, France [10]. The close phylogenetic relationship and potential for genetic introgression between these species suggest that *S. bovis* antigens might cross-react with antibodies generated against *S. haematobium* infections, offering a practical alternative diagnostic target.

Previous immunodiagnostic studies have shown that recombinant *S. bovis* antigens can be recognized by sera from livestock infected with schistosomes [11]. The *S. bovis* 22.6 kDa tegumental antigen shares high amino acid sequence identity with its *S. haematobium* ortholog, including conserved EF-hand calcium-binding motifs and dynein light-chain domains [11]. This structural similarity, combined with the successful use of *S. bovis* antigens for surveillance of ruminant schistosomiasis in European outbreak investigations [10], suggests its potential utility for human diagnostics.

We previously demonstrated the presence of anti-schistosomal antibodies in populations from Yewa North using crude parasite antigens [12]. In the present study, we evaluated whether the recombinant *S. bovis* 22.6 kDa antigen (r*Sb* 22.6 kDa) could detect anti-schistosomal antibodies in urine from individuals in *S. haematobium*-endemic communities and assessed its potential utility for serodiagnostic applications in this setting.

Materials and methods

Study design

This was a cross-sectional study conducted between October 2021 and March 2022 in four schistosomiasis endemic communities in Ogun State, Nigeria on the diagnostic performance of recombinant *S. bovis* 22.6 kDa antigen for detection of urinary schistosomiasis. Advocacy visits were made to the community (traditional rulers and school authorities) prior to data collection.

Study area and participants

The study was carried out on samples from individuals from the following four communities in Ogun State, Nigeria: Ibese, Eggua, Ijoun (Yewa North Local Government Area) and Imeko (Imeko-Afon Local Government Area) (Fig. 1). These are rural agrarian communities, which lie between latitude 7°26′55.97″N and longitude 2°50′34.40″E in a derived savannah zone. The area is largely dominated by Yoruba-speaking people with a significant mixing of people from the Republic of Benin [13]. These areas were selected due to the presence of a

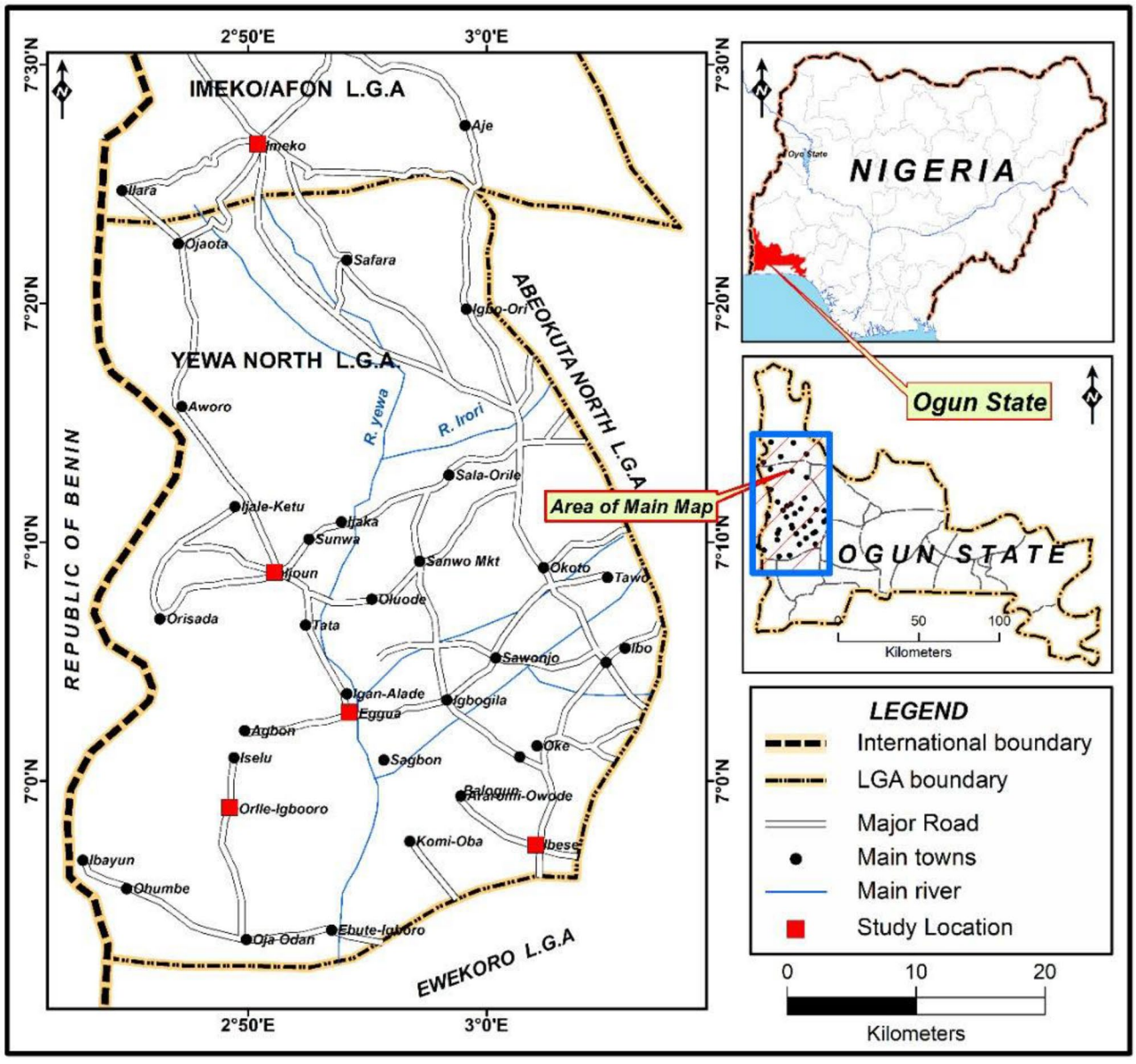

Fig. 1 Geographical map showing the study locations

flowing river (River Yewa) through the communities, which serves as main water source for religious, agricultural, and domestic purposes. Ethical clearance for the study was obtained in compliance with the approved ethical guidelines by the Ogun State Ministry of Health (HPRS/381/357). Voluntary participation was the principal norm; informed consent was obtained from each volunteer and assent was necessary for minors, in addition.

Sample size and sampling method

Sample size was calculated using the formula for cross-sectional studies [14].

$$n = (Z^2\alpha/2 \times p \times (1-p))/d^2$$

Where n is the required sample size, Zα/2 is 1.96 for 95% confidence level, p is the expected prevalence (0.25 based on previous studies in the region [18, 34]), and d is the desired margin of error (0.05). The calculation yielded: n = $(1.96^2 \times 0.25 \times 0.75)/0.05^2$ = 288 participants. Adjusting for an anticipated 10% non-response rate, the minimum required sample size was 317 participants. We employed a multistage sampling approach: purposive selection of communities based on proximity to water bodies, followed by systematic random sampling of households, and recruitment of all consenting household members aged ≥ 2 years.

Participant selection criteria

Participants were included in the study if they were aged 2 years or older, were permanent residents of the study communities, and had provided informed consent or assent. Individuals were excluded if they were unable to provide a urine sample, were extremely ill, or had received antischistosomal treatment within the previous three months. Withdrawal criteria were defined in advance as participant request or incomplete sample collection, although no withdrawals were recorded during the study.

Questionnaire survey

The questionnaire was adapted from a previous study (13) and administered individually in the local language (Yoruba). It was used to collect sociodemographic information, including name, age, sex, and occupation. In addition, information was obtained on symptoms such as pain during urination and abdominal pain, as well as behaviours that may facilitate disease transmission or influence treatment-seeking. Responses were first recorded on paper forms and subsequently entered into Microsoft Excel for analysis.

Collection of samples

Urine samples were collected in clean and labelled plastic universal bottles (20 mL) between the hours of 10:00 and 14:00 h from each participant. Samples were aliquoted in 2 mL Eppendorf tubes until further use. The samples were stored on ice or at 4 °C prior to transportation to the laboratory.

Urine examination for *Schistosoma haematobium* eggs

Each urine sample (10 mL) was centrifuged at 2,000 rpm for 5 min, and the sediment examined under a double eye Olympus microscope (Germany) using 10× and 40× objectives for *S. haematobium* egg identification and counting. *S. haematobium* eggs were identified by the presence of a terminal spine. Number of eggs was counted per 10 mL of urine, and the intensity of infection was determined as follows: 0 egg/10 mL of urine was reported as negative; 1–49 eggs/10 mL of urine as light intensity of infection; ≥50 eggs/10 mL of urine as heavy intensity of infection [15]. The rest of the urine was frozen immediately until further use.

Examination of urine for haematuria, proteinuria and leukocyturia

Urinalysis was done on the urine samples for the detection of blood and other analytes using reagent strips (Combi-11®Rapid Labs, UK). A urinalysis strip was gently dipped into the sterile sampling bottle containing the urine for 5s. The amount of blood, leukocytes, and protein in the urine was estimated by comparing the change in colour of the strip with the manufacturer's colour chart.

ELISA with recombinant *Schistosoma bovis* 22.6 kDa antigen (r*Sb* 22.6 kDa-ELISA)

An in-house indirect ELISA was performed following the protocol of Oleaga [10] with minor modifications. Briefly, flat-bottom 96-well microtiter plates (Sigma-Aldrich) were coated with 50 µL/well of r*Sb* 22.6 kDa antigen [11] at a concentration of 0.4 µg/well in 0.1 M carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C.

Plates were washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBST) and blocked with 200 µL/well of 3% bovine serum albumin (BSA) in PBS for 2 h at room temperature. After washing, urine samples were diluted 1:1 in 1% BSA-PBS (dilution buffer) and 50 µL was added to wells in triplicate. Control wells received dilution buffer only (blank controls). Plates were incubated for 2 h at room temperature, then washed three times with PBST.

Peroxidase-conjugated rabbit anti-human IgG (Nordic Immunological Laboratories, The Netherlands) diluted 1:4,000 in 1% BSA-PBS was added (50 µL/well) and plates were incubated for 1 h at room temperature. After final washing (three times with PBST), the reaction was developed by adding 50 µL/well of substrate solution containing 4 mg/mL ortho-phenylenediamine (Sigma-Aldrich) and 0.4 µL/mL H_2O_2 (Sigma-Aldrich) in citrate-phosphate buffer (pH 5.0). Plates were incubated for 10 min in the dark at room temperature, and the reaction was stopped with 50 µL/well of 3 N hydrochloric acid.

Optical density (OD) values were read at 492 nm using an ELISA microplate reader (MicroRead1000). All samples and controls were processed under the same assay conditions to ensure consistency. Each plate included the following controls: (1) a pooled positive control urine sample from confirmed *S. haematobium*-infected individuals; (2) three negative control samples from individuals with no history of schistosomiasis and negative microscopy; and (3) blank wells containing only dilution buffer and conjugate. These internal controls were included on every plate to ensure quality control and assay reliability.

The cut-off value for positivity was calculated as the mean OD of negative controls plus two standard deviations, with an additional 0.05 units added to account for estimated ELISA reader error [16] Cut-off = mean (OD negative controls) + 2SD + 0.05. Samples with OD values above this cut-off were classified as positive for the schistosomal 22.6KD antigen. Receiver operating characteristic (ROC) curve analysis was performed using ELISA optical density values as the test variable and microscopy result as the reference standard. The area under the curve

Table 1 Demographic characteristics of the study population

Characters	Total
Sex	
Male	235
Female	313
Missing data	11
Total	**559**
Age Group (Years)	
2–19	269
20–30	39
31–50	127
> 50	105
Missing data	19
Total	**559**
Community/Primary School	
Ibese Community	47
Imeko Community	28
Ijoun Community	95
Yewa North Primary School 1, Ijoun	136
Yewa North Primary School 3, Ijoun	64
Central Primary School, Eggua	113
Yewa Primary School, Eggua	76
Total	**559**
Occupation	
Civil Servant	68
Farming	45
Fashion Designing	22
Fishing	34
Food Vendor	19
Hairdresser	13
Students	86
Trading	155
Others	89
Missing data	28
Total	**559**

(AUC) with 95% confidence interval was calculated to assess the discriminatory performance of the assay.

Statistical analysis

Descriptive statistics were calculated for demographic variables and presented as frequencies and percentages. Prevalence estimates with 95% confidence intervals were calculated for both microscopy and ELISA results. Comparisons between proportions (ELISA vs. microscopy positivity; presence of clinical markers in ELISA-positive vs. ELISA-negative groups) were performed using Chi-square tests or Fisher's exact test where appropriate.

Diagnostic performance of the r*Sb* 22.6 kDa-ELISA was evaluated using microscopy as the reference standard, calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals. We acknowledge that microscopy based on a single urine sample has imperfect sensitivity and may underestimate true infection prevalence, which affects these calculations.

Correlation between ELISA optical density values and egg counts was assessed using Spearman's rank correlation coefficient. Associations between ELISA positivity and clinical parameters (haematuria, leukocyturia, proteinuria) were evaluated using logistic regression. All statistical analyses were performed using SPSS v29, and results were considered statistically significant at $P < 0.05$.

Results

Sociodemographic data

A total of 559 participants from Imeko, Ijoun, Ibese and Eggua villages were enrolled in this study. Of these participants, 558 provided urine samples. Twenty-eight (5.0%) participants were from Imeko, 47 (8.4%) from Ibese, 295 (52.8%) from Ijoun and 189 (33.8%) from Eggua. Two hundred and thirty-five (42.1%) were male and 313 (56.1%) were female. The sociodemographic characteristics of the participants are summarized in Table 1.

Urine microscopy analysis

Microscopic examination demonstrated the presence of *S. haematobium* eggs in the urine of 78 individuals (13.98%) from Imeko, Ijoun, Ibese and Eggua communities (Table 2). The egg counts ranged from 0 to 80/10 mL of urine. The geometric mean egg count was 27.5/10 mL of urine.

Table 2 Comparison between recombinant *Sb* 22.6 kDa ELISA and microscopy

Community/School	ELISA Results			Microscopy Results		
	Number examined	Positive *n* (%)	Negative *n* (%)	Number examined	Positive *n* (%)	Negative *n* (%)
Ibese	47	30 (63.8)	17 (36.2)	47	2 (4.3)	45 (95.7)
Imeko-Afon	28	18 (64.3)	10 (35.7)	28	1 (3.6)	27 (96.4)
Ijoun (Community & Schools)	295	125 (42.4)	170 (57.6)	294	38 (12.9)	256 (87.1)
Central Primary School, Eggua	113	60 (53.1)	53 (46.9)	113	14 (12.4)	99 (87.6)
Yewa Primary School, Eggua	70	66 (94.3)	4 (5.7)	76	23 (30.3)	53 (69.7)
TOTAL	**553**	**299 (54.1)**	**254 (45.9)**	**558**	**78 (14.0)**	**480 (86.0)**

$\chi^2 = 186.4$, $P < 0.001$ for comparison of ELISA vs. microscopy positivity

Table 3 Association between ELISA results and clinical markers

Parameter	ELISA Positive *n* = 299 n (%)	ELISA Negative *n* = 254 n (%)	Odds Ratio (95% CI)	*P*-value
Microhaematuria	165 (55.2)	62 (24.4)	3.79 (2.59–5.55)	< 0.001
Leukocyturia	265 (88.6)	136 (53.5)	6.84 (4.51–10.37)	< 0.001
Proteinuria	205 (68.6)	114 (44.9)	2.69 (1.89–3.83)	< 0.001
All three markers present	48 (16.1)	12 (4.7)	3.86 (1.99–7.50)	< 0.001
At least one marker present	198 (66.2)	176 (69.3)	0.87 (0.60–1.27)	0.473

Anti-schistosomal antibody detection by rSb 22.6 kDa ELISA *S. haematobium*

Anti-schistosomal reactivity with r*Sb* 22.6 kDa antigen was found in 299 (54.1%) individuals.

Screening with urinary ELISA using r*Sb* 22.6 kDa antigen showed that 30 (5.4%) of samples from Ibese, 18 (3.3%) from Imeko, 125 (22.6%) from Ijoun community and schools, 126 (22.8%) from Eggua (Community Primary School and Yewa North Primary School) were seropositive for anti-schistosomal antibodies (Table 2).

Association between ELISA and clinical markers from urine analysis

Among the 299 ELISA-positive individuals, 165 (55.2%) had microhaematuria, 265 (88.6%) had leukocyturia, and 205 (68.6%) had proteinuria (Table 3). In comparison, among ELISA-negative individuals, these proportions were 24.4% (62/254), 53.5% (136/254), and 44.9% (114/254) respectively.

ELISA-positive individuals had significantly higher odds of having microhaematuria (OR = 3.79, 95% CI: 2.59–5.55, $P < 0.001$), leukocyturia (OR = 6.84, 95% CI: 4.51–10.37, $P < 0.001$), and proteinuria (OR = 2.69, 95% CI: 1.89–3.83, $P < 0.001$) compared to ELISA-negative individuals.

Forty-eight individuals (16.1% of ELISA-positive samples) were positive for all three clinical markers (haematuria, leukocyturia, and proteinuria), a pattern consistent with urogenital pathology, though non-schistosomal causes cannot be excluded.

Among the 78 microscopy-positive individuals, 67 (85.9%) had at least one clinical marker present, compared to 198/299 (66.2%) of ELISA-positive individuals and 176/254 (69.3%) of ELISA-negative individuals.

Comparison between r*Sb* 22.6 kDa ELISA and microscopy

Of the 558 urine samples examined by microscopy, 78 (13.98%, 95% CI: 11.2–17.2%) were positive for *S. haematobium* eggs. In contrast, 299 of 553 samples tested by ELISA (54.1%, 95% CI: 49.9–58.2%) were positive for anti-schistosomal antibodies (Table 2). This difference was statistically significant ($\chi^2 = 186.4$, $P < 0.001$).

Among the 78 microscopy-positive individuals, only 36 (46.2%) were also ELISA-positive. Conversely, 221 of 480 microscopy-negative individuals (46.0%) were ELISA-positive. The distribution of test results by community is shown in Table 2, with the highest seropositivity rates observed in Yewa Primary School, Eggua (94.3%, 66/70) and Ijoun communities (42.4%, 125/295).

When single-sample microscopy was used as the reference standard, the diagnostic performance of the rSb 22.6 kDa ELISA was limited. All diagnostic performance indices with 95% confidence intervals are presented in Table 4. ROC curve analysis (Fig. 2) of ELISA optical density

Table 4 Diagnostic performance indices of the urine ELISA assay against microscopy

Diagnostic Index	Value (%)	95% Confidence Interval (CI)
Sensitivity	46.2	34.8 – 57.9
Specificity	45.8	41.4 – 50.3
Positive Predictive Value (PPV)	12.0	8.5 – 16.5
Negative Predictive Value (NPV)	83.5	78.3 – 87.8
Overall Accuracy	45.9	
Area Under the ROC Curve (AUC)	0.698	0.642 – 0.754

Legend: Values represent percentages accompanied by their corresponding 95% confidence intervals (CI), with the overall discriminatory performance summarized by the Area Under the Receiver Operating Characteristic curve (AUC)

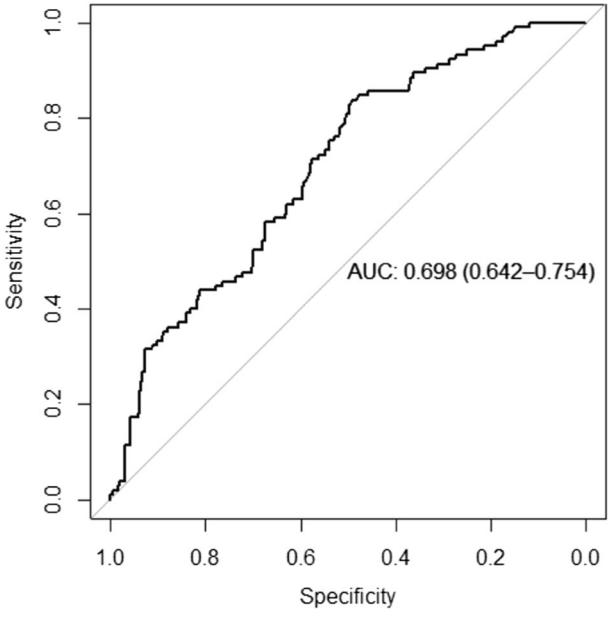

Fig. 2 Receiver operating characteristic (ROC) curve for the rSb 22.6 kDa ELISA using single-sample urine microscopy as the reference standard. The area under the curve (AUC) was 0.698 (95% CI: 0.642–0.754), indicating modest discriminatory performance

values against microscopy positivity yielded an AUC of 0.698 (95% CI: 0.642–0.754). The cut-off was determined as the mean OD of negative controls plus two standard deviations, with an additional 0.05 correction for reader error [16]; this approach was chosen to balance sensitivity against false-positive rates under field conditions.

Discussion

The primary finding of this study is that seropositivity to r*Sb* 22.6 kDa antigen (54.1%) was nearly four-fold higher than microscopy-detected egg prevalence (13.9%) in our study population. This discrepancy requires careful interpretation. It is important to emphasise at the outset that seropositivity in this context may reflect any of several possibilities: current low-intensity infection, past exposure with persistent antibodies, cross-reactivity with other parasites, or non-specific binding. Active infection cannot be inferred from seropositivity alone without corroborating diagnostic evidence.

First, antibody-based assays detect both active infections and past exposure with persistent antibodies. Immunological memory against schistosome antigens can persist for months to years after successful treatment or spontaneous clearance [17]. In our endemic communities where transmission is ongoing and treatment coverage varies, many individuals may have antibodies from previous infections even if they are currently egg-negative [18]. This is supported by the fact that 11 participants self-reported previous schistosomiasis diagnosis, though the actual number with prior infection is likely much higher.

Second, our use of a single urine sample for microscopy likely underestimated true infection prevalence. *Schistosoma haematobium* egg excretion varies day-to-day and peaks around midday [19]. Standard protocols recommend examining three consecutive daily samples to achieve adequate sensitivity, which was not feasible in our field setting. Studies have shown that single-sample microscopy detects only 60–70% of infections compared to three-sample protocols [5]. Therefore, some of the 221 individuals who were microscopy-negative but ELISA-positive may have low-intensity active infections that were missed by single-sample examination.

Third, the high seropositivity may reflect intense ongoing transmission in these communities, with many individuals experiencing repeated low-level exposures that generate antibody responses but insufficient worm burdens to produce detectable eggs [1]. The significant association between ELISA positivity and clinical markers of urogenital pathology is consistent with this interpretation, though these markers are not specific to schistosomiasis and cannot alone confirm active infection.

However, we must also consider the possibility of false-positive ELISA results. The relatively low specificity (41.7%) when using microscopy as reference is concerning, though this must be interpreted cautiously given microscopy's own sensitivity limitations. Cross-reactivity with other parasites, non-specific binding, or residual antibodies from treated infections could all contribute to positive ELISA results in truly uninfected individuals [20].

Cross-species reactivity and implications for hybridization

The successful detection of antibodies using *S. bovis* antigen in *S. haematobium* infected people is consistent with cross-reactivity between these species [21]. The high sequence conservation between species of the 22.6 kDa protein [11] suggests that the protein plays an essential functional role and contains immunodominant epitopes shared across species [22].

While our study did not include molecular characterization to detect actual *S. haematobium* × *S. bovis* hybrids, the documented presence of such hybrids in neighboring West African countries [7, 8] and their role in transmission to humans suggests they may also occur in Nigeria. The presence of cattle and other ruminants in our study communities, along with shared *Bulinus* snail habitats, creates ecological conditions favorable for hybridization. Future studies incorporating molecular diagnostics (e.g., mitochondrial and nuclear gene sequencing) are needed to determine whether hybrids contribute to transmission in these Nigerian communities.

Another possibility could be that some ELISA-positive, microscopy-negative individuals harbour *S. haematobium* × *S. bovis* hybrid infections [23] and may exhibit reduced fecundity or produce non-viable eggs, resulting in antibody responses without detectable egg excretion. This could partially explain the high seropositivity rates observed in our study. From a diagnostic perspective, if hybridization is occurring, the use of *S. bovis* antigens becomes even more relevant. Hybrid parasites may express antigens from both parental species, making cross-reactive diagnostic tools more appropriate than species-specific assays [24].

Clinical correlates and morbidity assessment

The strong association between ELISA positivity and urinary pathology markers (Microhaematuria, leukocyturia, and proteinuria) are well-established consequences of *S. haematobium* egg deposition in the bladder and urinary tract [25]. However, we must acknowledge that these markers are not specific to schistosomiasis. Bacterial infections in the urinary tract infections, urolithiasis, and chronic kidney disease can produce similar findings [26]. Indeed, co-infection of *S. haematobium* with bacterial pathogens has been documented [27, 28], and the inflammatory environment created by schistosome eggs may predispose to secondary bacterial infections.

The relatively high prevalence of proteinuria (37.1% overall) is of particular concern, as it may indicate glomerular damage. Previous work in Eggua community documented elevated kidney pathology markers in schistosomiasis-endemic populations [13]. The contribution of *APOL1* gene variants, which are common in West African populations and associated with kidney disease risk [29], warrants further investigation in the context of schistosome-associated renal pathology.

Diagnostic performance and utility

The diagnostic performance metrics of rSb 22.6 kDa-ELISA (sensitivity 46.2%, specificity 45.8%) indicate that it cannot serve as a standalone diagnostic test for individual case detection. This is further supported by the ROC curve analysis: the AUC of 0.698 indicates moderate discriminatory ability, below the threshold typically required for clinical diagnostics. However, this assessment depends entirely on accepting single-sample microscopy as a reliable reference standard, which it is not.

The true diagnostic utility of this ELISA may lie not in individual diagnosis but in population-level applications. The assay could be useful for mapping transmission intensity across geographical areas [30]. High seropositivity rates may indicate areas with intense ongoing transmission requiring intervention, even if current egg prevalence appears low. This is analogous to the use of antibody-based assays for mapping malaria transmission in low-endemic areas [31]. Serial seroprevalence measurements could track the impact of mass drug administration (MDA) programs over time. While antibodies persist after treatment, declining seropositivity rates in young children (who represent recent transmission) could indicate successful interruption of transmission [32].

In research settings, our ELISA could serve as an initial screening tool to identify samples warranting more expensive molecular diagnostics (PCR, loop-mediated isothermal amplification) [33]. The high negative predictive value (83.5%) suggests that ELISA-negative samples are unlikely to be positive by other methods. The 221 microscopy-negative but ELISA-positive individuals represent an important group for further investigation. Without additional confirmatory diagnostic methods, such as molecular or circulating antigen assays, it is not possible to determine whether these individuals represent low-intensity infections missed by microscopy, previous exposure with persistent antibodies, cross-reactivity, or non-specific serological responses. Further studies are therefore needed to clarify the epidemiological significance of these findings.

Comparison with other studies and prevalence context

Our microscopy-detected prevalence of 13.9% is lower than previous reports from the same region: Adewoga [34] found 48.4% prevalence in Yewa North, and Bakare [15] reported 21.3% in Eggua community. Several factors may explain the differences observed between our findings and those of earlier studies. Temporal variation is one possible contributor, as our study was conducted in 2021–2022, several years after previous surveys. During this period, ongoing mass drug administration (MDA) efforts may have reduced infection prevalence, although the presence of infected individuals indicates that transmission is still taking place in these communities. Geographical variation may also play a role, particularly given the low egg counts recorded in Imeko and Ibese communities, which accounted for 13.4% of our total sample. These areas are located on higher ground and are less dependent on river water sources; most residents reported using wells and boreholes, which likely reduces exposure to infected water and therefore lowers prevalence compared to communities with access to rivers.

Methodological differences may further contribute to these discrepancies. Factors such as single-sample collection, time of day of urine collection, and the smaller volume examined (10 mL compared to larger volumes in some studies) all reduce the sensitivity of egg detection [35]. The high seropositivity alongside low egg prevalence may therefore indicate that transmission intensity has declined from previously higher levels, leaving a population with residual antibodies from past infections. Alternatively, it could reflect a shift toward low-intensity or subpatent infections that remain immunologically active but are less likely to be detected by microscopy.

Study limitations

Several limitations must be considered when interpreting our findings. The most significant is the collection of only a single urine sample per participant, which may have reduced the sensitivity of microscopy and made it an imperfect reference standard for assessing ELISA performance [20]. Without multi-sample microscopy, PCR, or circulating antigen assays, the true sensitivity and specificity of the ELISA remain uncertain. Another limitation concerns antibody persistence, as this study could not distinguish between active infections and past exposures.

The rSb 22.6 kDa antigen could cross-react with antibodies against other schistosome species, including *S. bovis-haematobium* hybrids or ectopic *S. mansoni* infections. While *S. mansoni* eggs are rarely found in urine, co-infections in these communities cannot be ruled out. Future studies should include species-specific molecular diagnostics to determine whether observed seropositivity represents exposure to *S. haematobium* alone or

a broader range of schistosome species present in the region.

In this study, we did not screen for co-infections with soil-transmitted helminths or trematodes that might cross-react with the *S. bovis* antigen. This could potentially affect the specificity of the assay and contribute to false-positive results. Future studies should include comprehensive parasitological screening to assess cross-reactivity patterns.

The absence of molecular confirmation is a further limitation: PCR or antigen-detection assays could have resolved the diagnostic status of ELISA-positive, microscopy-negative individuals, and cross-reactivity with co-endemic helminths was not systematically assessed. The lack of treatment follow-up also prevents evaluation of whether antibody levels decline after praziquantel — which would clarify whether seropositivity tracks current or historical exposure. Finally, some antibody degradation during sample transport cannot be excluded [36]. These gaps define the priorities for future work: multi-method diagnostic validation, post-treatment serology, and molecular characterisation of local schistosome populations to better define the epidemiological value of this assay.

Broader implications

These findings have implications beyond the diagnostic question. Control programmes relying solely on egg-based microscopy risk underestimating ongoing transmission, which is particularly relevant as Nigeria moves toward WHO 2030 elimination targets. The cross-reactivity of a cattle-derived antigen in this human population also underscores the value of a One Health framework: integrating livestock surveillance for *S. bovis* and monitoring hybridization events could strengthen understanding of transmission ecology and inform more effective, cross-sectoral control strategies.

Conclusion

This study demonstrates that the recombinant *S. bovis* 22.6 kDa antigen successfully cross-reacts with antibodies in the urine of individuals from *S. haematobium*-endemic communities in Nigeria. The assay revealed a high seroprevalence (54.1%) that was strongly associated with clinical markers of urogenital pathology (hematuria, leukocyturia, and proteinuria), contrasting sharply with the low egg-detection rate (13.9%) via microscopy. While this elevated seropositivity likely captures low-intensity infections missed by conventional microscopy, it may also reflect persistent post-exposure antibodies or species cross-reactivity rather than active disease.

Consequently, with a sensitivity of 46.2% and a specificity of 45.8%, this Schisto bovis urine ELISA is inadequate for individual clinical diagnosis in our hands. However, it may have potential as a non-invasive, population-level surveillance tool for seroprevalence mapping, transmission intensity tracking, and monitoring control program impacts—particularly in low-endemicity zones where microscopy lacks sufficient sensitivity. Furthermore, the robust cross-reactivity between *S. bovis* and *S. haematobium* antigens highlights the utility of the assay within a One Health framework, offering valuable insights into potential species hybridization and the development of pan-schistosome diagnostic tools. With further optimization and clearer understanding of what antibody positivity represents in different epidemiological contexts, this approach could contribute to improved surveillance and control of urogenital schistosomiasis in endemic regions.

Abbreviations

APOL1	Apolipoprotein L1
ARNTD	African Research Network for Neglected Tropical Diseases
BSA	Bovine Serum Albumin
COR-NTD	Coalition for Operational Research on Neglected Tropical Diseases
CSIC	*Consejo Superior de Investigaciones Científicas* (Spanish National Research Council)
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
EF-hand	Helix–loop–helix structural motif (calcium-binding domain)
ELISA	Enzyme-Linked Immunosorbent Assay
HCl	Hydrochloric Acid
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IIS La Fe	*Instituto de Investigación Sanitaria La Fe* (Health Research Institute La Fe, Valencia, Spain)
IRNASA	Institute of Natural Resources and Agrobiology, Salamanca, Spain
L.G.A.	Local Government Area
MDA	Mass Drug Administration
OD	Optical Density
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline with Tween-20
PCR	Polymerase Chain Reaction
rSb 22.6 kDa	Recombinant *Schistosoma bovis* 22.6 Kilodalton Antigen
Rpm	Revolutions Per Minute
SD	Standard Deviation
UK aid	United Kingdom International Development Aid
USAID	United States Agency for International Development

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Author contributions

CIA, AM, AO.: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Data interpretation, Writing - original draft, Writing - review & editing. TA, CA, RE, OO, MT, OO, HO, CIA.: Investigation, Field coordination, Parasitological analysis, Data curation, Writing - review & editing. AO, ME, OO, CIA, AM.: Methodology, Investigation, ELISA assays, Data curation. CA, OO, ME, CIA.: Investigation, Laboratory support, Sample processing, Resources. CIA, TA, CA, RE.: Formal analysis, Data visualization, Statistical analysis. All: Writing - original draft, review and editing, Critical review of intellectual content.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was given by the by the State Health Research Ethics Committee of the Ogun State Ministry of Health, Nigeria; HPRS/381/357. Informed consent was obtained from all the participants and from the legal guardians of the participants who were below 16 years of age.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Colley DG, Bustinduy AL, Secor WE, King CH. Hum Schistosomiasis Lancet. 2014;383(9936):2253–64.
2. Houmsou RS, Wama EB, Agere H, Uniga JA, Bingbeng JB, Jerry JT, et al. Integrated control of river and pond water as an exposure source to urogenital schistosomiasis of rural inhabitants in southern Taraba State, Nigeria. J Water Sanit Hyg Dev. 2021;11(4):546–557. Available from: https://iwaponline.com/washdev/article/11/4/546/81582/Integrated-control-of-river-and-pond-water-as-an.
3. Sowole A, Adegbite A. Correlation analysis between the prevalence of *Schistosoma haematobium* and water conditions: a case study among school pupils in southwestern Nigeria. Int J Recent Res Appl Stud. 2012;13(1):160–5.
4. Anumudu C, Onile O, Awobode H, Tokunbo A, Oladele V, Adebayo A, et al. Social, Cultural and Environmental determinants of a Proposed Schistosomiasis Health Education Intervention in Eggua, Nigeria. J Behav Health. 2019;8(1):1. https://doi.org/10.5455/jbh.20190831052526.
5. Ochodo EA, Gopalakrishna G, Spek B, Reitsma JB, van Lieshout L, Polman K, et al. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database Syst Rev. 2015;2015(3):CD009579. https://doi.org/10.1002/14651858.CD009579.pub2.
6. Silva-Moraes V, Shollenberger LM, Castro-Borges W, Rabello A, Harn DA, Medeiros L, et al. Serological proteomic screening and evaluation of a recombinant egg antigen for the diagnosis of low-intensity *Schistosoma mansoni* infections in endemic area in Brazil. PLOS Negl Trop Dis. 2019;13(3):e0006974. https://doi.org/10.1371/journal.pntd.0006974.
7. Pennance T, Allan F, Emery A, Rabone M, Cable J, Garba AD, et al. Interactions between *Schistosoma haematobium* group species and their *Bulinus* spp. intermediate hosts along the Niger River Valley. Parasites Vectors. 2020;13(1).
8. Fall CB, Lambert S, Léger E, Yasenev L, Garba AD, Diop SD, Borlase A, Catalano S, Faye B, Walker M, Sene M, Webster JP. Hybridized Zoonotic *Schistosoma* infections result in hybridized morbidity profiles: A clinical morbidity study amongst co-infected human populations of Senegal. Microorganisms. 2021;9(8):1776. https://doi.org/10.3390/microorganisms9081776. PMID: 34442855; PMCID: PMC8401530.
9. Angora EK, Allienne JF, Rey O, Menan H, Touré AO, Coulibaly JT, et al. High prevalence of *Schistosoma haematobium* × *Schistosoma bovis* hybrids in schoolchildren in Côte d'Ivoire. Parasitology. 2019;147(3):287–94. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10317610/.
10. Oleaga A, Rey O, Polack B, Grech-Angelini S, Quilichini Y, Pérez-Sánchez R, et al. Epidemiological surveillance of schistosomiasis outbreak in Corsica (France): Are animal reservoir hosts implicated in local transmission? PLOS Negl Trop Dis. 2019;13(6):e0007543. https://doi.org/10.1371/journal.pntd.0007543.
11. De la Torre-Escudero E, Manzano-Román R, Pérez-Sánchez R, Barrera I, Siles-Lucas M, Oleaga A. Molecular cloning, characterization and diagnostic performance of the Schistosoma bovis 22.6 antigen. Vet Parasitol. 2012;190(3–4):530–40. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0304401712003202.
12. Anumudu C, Alabi O, Oniya M. Schistosome specific antibodies in individuals co-infected with malaria in southwest Nigeria. Niger J Parasitol. 2012;33(2). Available from: https://www.ajol.info/index.php/njpar/article/view/99753.
13. Onile OS, Calder B, Soares NC, Anumudu CI, Blackburn JM. Quantitative label-free proteomic analysis of human urine to identify novel candidate protein biomarkers for schistosomiasis. PLOS Negl Trop Dis. 2017;11(11):e0006045. https://doi.org/10.1371/journal.pntd.0006045.
14. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench. 2013;6(1):14–17. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4017493/.
15. Bakare SO, Adebayo AS, Awobode HO, Onile OS, Anumudu CI. Arsenicosis in bladder pathology and schistosomiasis in Eggua, Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2018;112(5). Available from: https://www.researchgate.net/publication/325604747_Arsenicosis_in_bladder_pathology_and_schistosomiasis_in_Eggua_Nigeria.
16. Chen R, Shang H, Niu X, Huang J, Miao Y, Sha Z, et al. Establishment and evaluation of an indirect ELISA for detection of antibodies to goat Klebsiella pneumonia. BMC Vet Res. 2021;17(1).
17. Molehin AJ. Current Understanding of Immunity Against Schistosomiasis: Impact on Vaccine and Drug Development. Res Rep Trop Med. 2020;11:119–28. https://doi.org/10.2147/RRTM.S225746.
18. Kulinkina AV, Kosinski KC, Adjei MN, Osabutey D, Gyamfi BO, Biritwum NK, et al. Contextualizing *Schistosoma haematobium* transmission in Ghana: Assessment of diagnostic techniques and individual and community water-related risk factors. Acta Trop. 2019;194:195–203.
19. Doehring E, Feldmeier H, Daffalla AA. Day-to-day variation and circadian rhythm of egg excretion in urinary schistosomiasis in the Sudan. Ann Trop Med Parasitol. 1983;77(6):587–94. Available from: https://pubmed.ncbi.nlm.nih.gov/6660966/.
20. Doenhoff MJ, El-Faham MH, Liddell S, Fuller HR, Stanley RG, Schramm G, et al. Cross-reactivity between Schistosoma mansoni antigens and the latex allergen Hev b 7: putative implication of cross-reactive carbohydrate determinants (CCDs). PLoS ONE. 2016;11(7):e0159542. https://doi.org/10.1371/journal.pone.0159542.
21. Agnew AM, Murare HM, Lucas SB, Doenhoff MJ. *Schistosoma bovis* as an immunological analogue of S. haematobium. Parasite Immunol. 1989;11(4):329–40. Available from: https://pubmed.ncbi.nlm.nih.gov/2506507/.
22. Carvalho-Queiroz C, Cook R, Wang CC, Correa-Oliveira R, Bailey NA, Egilmez NK, et al. Cross-reactivity of Schistosoma mansoni cytosolic superoxide dismutase, a protective vaccine candidate, with host superoxide dismutase and identification of parasite-specific B epitopes. Infect Immun. 2004;72(5):2635–47. https://doi.org/10.1128/IAI.72.5.2635-2647.2004.
23. Teukeng FFD, Blin M, Bech N, Gomez MR, Zein-Eddine R, Simo AMK, et al. Hybridization increases genetic diversity in Schistosoma haematobium populations infecting humans in Cameroon. Infect Dis Poverty. 2022;11(1).

24. King KC, Stelkens RB, Webster JP, Smith DF, Brockhurst MA. Hybridization in Parasites: Consequences for Adaptive Evolution, Pathogenesis, and Public Health in a Changing World. PLOS Pathog. 2015;11(9):e1005098. https://doi.org/10.1371/journal.ppat.1005098.
25. Osakunor D, Ishida K, Lamanna OK, Rossi M, Dwomoh L, Hsieh MH. Host tissue proteomics reveal insights into the molecular basis of Schistosoma haematobium-induced bladder pathology. PLOS Negl Trop Dis. 2022;16(2):e0010176. https://doi.org/10.1371/journal.pntd.0010176 Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8846513/.
26. Aeddula N, Vaidya S. Chronic kidney disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535404/.
27. Ossai OP, Dankoli R, Nwodo C, Tukur D, Nsubuga P, Ogbuabor D, et al. Bacteriuria and urinary schistosomiasis in primary school children in rural communities in Enugu State, Nigeria, 2012. Pan Afr Med J. 2014;18(Suppl 1):15. https://doi.org/10.11604/pamjs.supp.2014.18.1.4169.
28. Kone KJ, Onifade AK, Dada EO. Occurrence of urinary schistosomiasis and associated bacteria in parts of Ondo State, Nigeria. PLOS Glob Public Health. 2022;2(10):e0001119. https://doi.org/10.1371/journal.pgph.0001119.
29. Adu D. Contribution of apolipoprotein L1 (APOL1) risk alleles to kidney disease in West Africa: an opportunity for treatment. Ghana Med J. 2025;58(4):249–50. Available from: https://www.ajol.info/index.php/gmj/article/view/285614.
30. Soe HZ, Oo CC, Myat TO, Maung NS. Detection of Schistosoma Antibodies and exploration of associated factors among local residents around Inlay Lake, Southern Shan State. Myanmar Infect Dis Poverty. 2017;6(1).
31. Macalinao MLM, Fornace KM, Reyes RA, Hall T, Bareng APN, Adams JH, et al. Analytical approaches for antimalarial antibody responses to confirm historical and recent malaria transmission: an example from the Philippines. Lancet Reg Health West Pac. 2023;37:100792. https://doi.org/10.1016/j.lanwpc.2023.100792.
32. Amarir F, Abdelaali B, Abderrahim S, Bachir AE, Mohamed R. Survey and Diagnostic Challenges after Transmission-Stop: Confirming Elimination of *Schistosomiasis haematobium* in Morocco. J Parasitol Res. 2020;2020:1–7. https://doi.org/10.1155/2020/4591079.
33. Mei SJ, Yeap SK, Omar AR, Tan SW. Application of PCR-ELISA in molecular diagnosis. BioMed Res Int. 2014;2014:1–6. Available from: https://www.hindawi.com/journals/bmri/2014/653014/.
34. Adewoga TOS, Akinboade OA, Emikpe BO, Morenikeji O, Sobande AI. Prevalence and intensity of urinary schistosomiasis in school-age children in Yewa North Local Government Area of Ogun State, Nigeria. Annu Res Rev Biol. 2019;34(6):1–6.
35. Dolscheid-Pommerich RC, Klarmann-Schulz U, Conrad R, Stoffel-Wagner B, Zur B. Evaluation of the appropriate time period between sampling and analyzing for automated urinalysis. Biochemia Medica. 2016;26(1):82–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4783094/#r1
36. World Health Organization. Collection, storage and shipment of specimens for laboratory diagnosis and interpretation of results. Geneva: World Health Organization. 2012. Available from: https://www.ncbi.nlm.nih.gov/books/NBK143256/.

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