

Comparison of parasitological, serological and molecular diagnostic methods for schistosomiasis japonica: insights into the detection of light infections

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Abstract

Background

Accurate diagnosis of *Schistosoma japonicum* infection is essential for surveillance and elimination efforts, particularly in settings where infection intensities are low due to the implementation of mass drug administration (MDA). This study evaluated the diagnostic performance and operational characteristics of five diagnostic methods for detecting *S. japonicum* infection in the Philippines.

Methods

A cross-sectional study was conducted in Ekiran, Alangalang, Leyte Province, Philippines, in 2022. Five diagnostic methods were evaluated, including the Kato–Katz (KK) technique, two copro-DNA-based quantitative real-time PCR (qPCR) assays, and two serological assays, namely an enzyme-linked immunosorbent assay (ELISA) and a lateral flow immunoassay (LFIA), using samples collected from study participants. Diagnostic performance was assessed against both the KK and a composite reference, as well using latent class analysis (LCA). Operational characteristics, including sample requirements, time, cost, equipment and personnel training needs, and field applicability, were compared.

Results

The positivity rates estimated by the KK, qPCR1, qPCR2, ELISA and LFIA were 25.1% (137/545), 25.9% (141/545), 29.9% (163/545), 45.3% (247/545) and 48.8% (266/545), respectively, whereas the composite reference indicated an overall prevalence estimate of 34.5% (188/545). Compared with the composite reference, KK achieved the highest Youden's index (0.729), followed by qPCR2 incorporating the NaOH-based DNA extraction method (0.721), indicating good discriminatory performance. The serological assays demonstrated the highest sensitivities (83.0–84.0%) but moderate specificities (69.2–75.1%). In the two-class LCA model, the two molecular tests showed model simulated sensitivities and specificities exceeding 90%, whereas the serological assays showed relatively high sensitivities (~ 90%) but moderate specificities (65.3–70.5%). In terms of cost, testing time and logistical convenience, the LFIA was the most rapid and field-suitable test for onsite screening.

Conclusions

This comprehensive comparison further defined the diagnostic performance and features for each assay. The choice of diagnostic tool should be considered in relation to the intended purpose of testing and the local epidemiological and programmatic context, taking into account diagnostic performance as well as practical considerations such as cost, infrastructure requirements, and field applicability.

Background

Schistosomiasis is a debilitating and potentially life-threatening parasitic disease that affects more than 250 million people worldwide (1, 2). In the Philippines, schistosomiasis caused by the infection of *Schistosoma japonicum*, remains a major public health concern. The disease is prevalent in 28 provinces across 12 regions in the country, with approximately 12 million people at risk of infection, resulting in an estimated 22,300 disability-adjusted life years (DALYs) lost annually (3, 4). High-burden infection of the parasite can cause severe hepatosplenic complications and the disease is associated with anemia, malnutrition, and impaired cognitive development in children (5, 6). As a zoonotic disease, *S. japonicum* can infect more than 40 mammalian species as reservoir hosts, including bovines and rodents (7). Innovative integrated control strategies, including the implementation of cost-effective surveillance systems, environmental management, and interventions targeting animal reservoirs, such as replacing bovines with tractors for farming, have been proposed as components for achieving schistosomiasis elimination in the Philippines (3, 4, 8–10).

Diagnostic methods for schistosomiasis can be broadly classified into four categories: parasitological, serological, urine-based, and molecular tests (11, 12). Stool examination using the Kato–Katz (KK) technique is a widely used method and considered the first-line diagnostic approach for intestinal schistosomiasis. However, its sensitivity is often insufficient in the post–mass drug administration (MDA) era, where endemicity of schistosome infection has declined (13, 14). Common serological methods, which include antibody detection (AbD)-based and antigen detection (AgD)-based assays, are widely used for screening and surveillance. Serological techniques include enzyme-linked immunosorbent assay (ELISA) (15–18), indirect hemagglutination assay (IHA) (19) and point-of-care (POC) lateral flow assays (LFAs) (20–22). These methods generally provide high sensitivity and are useful screening tools; however, they often have limited ability to distinguish between past and active infections. Among urine-based diagnostics, the point-of-care circulating cathodic antigen (POC-CCA) assay is widely used for the detection of *Schistosoma mansoni* infection, particularly in areas of moderate to high endemicity (23). However, the assay has limitations such as cross-reactivity, low specificity, and issues with interpreting ‘Trace’ results (24, 25). Another urine-based test, up-converting phosphor-lateral flow circulating anodic antigen (UCP-LF-CAA) assay, can detect both *S. mansoni* and *S. haematobium* infection with mediocre diagnostic performance (26, 27). Compared with POC-CCA, UCP-LF-CAA, is laboratory-dependent, requiring specialized equipment, protocols, and technical expertise, which limits its suitability for POC application (28). Molecular techniques include polymerase chain reaction (PCR)-based technologies such as quantitative PCR (qPCR) (29, 30) and droplet digital PCR (ddPCR) (31, 32), as well as isothermal amplification techniques, such as loop-mediated isothermal amplification (LAMP) (33–35) and recombinase polymerase amplification (RPA) (36–40). These techniques usually exhibit high performance and are increasingly used in schistosomiasis diagnosis. However, their widespread implementation in large-scale surveillance programs remains constrained by higher costs, specialized equipment requirements, and the need for trained personnel. In the absence of a universally accepted gold-standard diagnostic test for schistosomiasis, comprehensive evaluation of available diagnostic approaches is essential (16, 27, 41–44). Such assessments can improve understanding of the strengths and limitations of individual approaches and support the selection of appropriate tools for surveillance, control, and elimination programs.

We previously conducted a cross-sectional study in a schistosomiasis endemic village, Ekiran, Alangalang, Leyte Province, Philippines (20, 45). ID matched stool and serum samples were collected from a human cohort during the study. These samples were used to validate different diagnostic assays. For example, sera were used for testing two serological assays: an in-house ELISA targeting the saposin protein, SjSAP4 and a lateral

flow immunoassay (LFIA) incorporating SjSAP4 (20). We also developed two copro-DNA-*SjTR1*-qPCR systems: a Maxwell kit-based copro-DNA-*SjTR1*-qPCR system (referred to as *SjTR1*-qPCR-S1 or qPCR1) and a NaOH-based copro-DNA-*SjTR1*-qPCR system (referred to as *SjTR1*-qPCR-S2 or qPCR2) for the detection of schistosome-derived DNA in stool samples (45).

The present study aimed to compare the diagnostic performance of five methods – the KK, two copro-DNA-qPCR assays (qPCR1 and qPCR2) (45), ELISA, and LFIA (20)– for the detection of *S. japonicum* infection, in parallel. In addition, we evaluated key operational characteristics of these diagnostic approaches, including equipment requirements, cost, processing time, and suitability for field deployment. Our findings provide valuable insights into the strengths and limitations of different diagnostic methods for detecting *S. japonicum* infections and may help inform the selection of appropriate tools for schistosomiasis surveillance and control programs.

Methods

Study site, human cohort and sampling

The experiments and analyses presented in this study were conducted as a part of a collaborative e-Asia joint program aimed at developing innovative diagnostic and environmental monitoring tools for schistosomiasis control in the Philippines. Data and clinical samples were collected in October 2022 from participants residing in Ekiran village, Alangalang, Leyte Province, the Philippines. This region is characterised by tropical rainforest/monsoon climate, with high annual rainfall, extensive river systems, and numerous freshwater bodies that provide conducive habitats for intermediate host snails. A total of 599 participants were enrolled in the study (20). Each participant was asked to provide three stool samples over a one-week period. For serological analyses, 10 ml of venous blood was collected from each participant, except for children aged 2–5 years, from whom 1–2 mL was collected. The blood samples were allowed to clot for at least 30 min, and were subsequently centrifuged at $1,500 \times g$ for 10 min. The resulting serum was aspirated, aliquoted into 2 ml cryotubes, and transported to QIMR Berghofer on dry ice for further serological analysis. The stool samples collected on the first day were preserved in 80% ethanol and transported to QIMR Berghofer, Australia, at ambient temperature, for DNA extraction and molecular analysis.

Parasitological examinations

The presence of parasite eggs in the stool samples was determined using the KK method (Fig. 1) (20). For each stool sample, laboratory staff prepared duplicate KK slides, which were analysed by trained microscopists using a light microscope at RITM in Muntinlupa, the Philippines. The KK findings were reported as eggs per gram (EPG) of faeces, calculated by multiplying the average egg numbers observed in six smears by 20 (Table S1). To verify the KK results, 10% of slides were randomly re-evaluated by skilled microscopists.

Serological tests

The in-house developed serological assays, SjSAP4-ELISA and SjSAP4-incorporated LFIA (Fig. 1), were evaluated using the serum samples collected from Ekiran, Alangalang (20). The LFIA results were captured using an iPhone 8 at 25 min after sample loading. All images were transferred to a computer, and intensity profile plots of the test images were generated using ImageJ 1.53 to quantify the intensities of the developed

test (T) and control (C) lines. Quantitative analysis was performed by calculating the T/C ratios. The resulting data were retrieved for comparison in the present study.

qPCR-based molecular tests

Two copro-DNA-qPCR systems were previously developed for the detection of *S. japonicum* infection by incorporating Maxwell kit-based (*SjTR1*-qPCR-S1 or qPCR1) and NaOH-based (*SjTR1*-qPCR-S2 or qPCR2) DNA extraction (Fig. 1) (45). Each qPCR assay was performed in triplicated. A qPCR result was considered positive if all the following criteria were met: 1) at least two of the three wells showed amplification; 2) the mean Ct value was < 35.143; 3) the standard deviation of the Ct values was < 1. The qPCR operators were blinded to the KK and serology results. The results were retrieved for re-analysis and comparison in this study.

Latent class analysis (LCA)

We fitted penalized-likelihood latent class models with one to three classes to the binary KK, qPCR1, qPCR2, ELISA and LFIA results from 545 participants, without designating a perfect reference test (46). The two-class log-linear model included a common within-class ELISA–LFIA association, whereas other test pairs were conditionally independent (47). The three-class model assumed conditional independence as its additional class directly represented a pattern in which ELISA and LFIA were commonly positive, whereas KK, qPCR1 and qPCR2 were predominantly negative. Classes were labelled after estimation as Negative and Positive in the two-class model and Negative, Intermediate and Positive in the three-class model; these labels describe test-result patterns rather than confirmed infection states. A preliminary unpenalized three-class fit produced a boundary estimate and unstable Hessian. We therefore applied weak symmetric smoothing equivalent to 0.5 pseudo-negative and 0.5 pseudo-positive observations per class and test (48).

Models were fitted in R 4.4.2 from 500 starting values initialized using poLCA 1.6.0.2 (49). Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) used the observed-data likelihood at the penalized estimates. Entropy, class size, interpretability and diagnostic checks were also considered. Approximate 95% Wald CIs were calculated on the logit scale using standard errors derived from the penalized Hessian and then back-transformed to the probability scale. In the two-class model, sensitivity was defined as P(test positive | Positive profile) and specificity as P(test negative | Negative profile). The three-class model was interpreted using the model-estimated probability of a positive result for each test within each latent class. Residual diagnostics for the two-class model suggested remaining within-class association between KK and ELISA. A sensitivity model therefore included a common KK–ELISA dependence term. Since the estimates and substantive conclusions were materially unchanged, the more parsimonious model was retained as the final model.

Statistical analysis

All diagnostic variables were harmonized and recorded into binary format for analysis, with no missing or indeterminate results. All statistical analyses were performed using GraphPad Prism version 10.4.1 (GraphPad software, San Diego, CA, USA) and Microsoft Excel 365. Age smoothing curves of *S. japonicum* prevalence stratified by sex were generated in RStudio (v2024.04.2 + 764; R v.4.4.1) using locally estimated scatterplot

smoothing (LOESS) implemented in ggplot2 package. A composite reference was defined to classify participant as positive based on any of the following test results: 1) a positive KK test ($n = 137$), 2) a negative KK test with positive results from both copro-DNA-qPCR systems ($n = 36$); or 3) a negative KK test, a positive result from one copro-DNA-qPCR assay, and a positive SjSAP4-ELISA result ($n = 15$). Difference in infection intensity between male and female participants was assessed using the Mann-Whitney U test. The differences in prevalence determined by the different methods were evaluated using the McNemar's chi-square (χ^2_m) test (<https://www.graphpad.com/quickcalcs/mcnemar1/>). Differences in *S. japonicum* positivity rates between sexes were evaluated using the Chi-square (χ^2) test for each diagnostic methods and the composite reference.

Diagnostic performance metrics, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's J statistic, were calculated by calibrating with the combined reference. Agreement between each diagnostic method and the composite reference standard was assessed using Cohen's kappa coefficient (κ) (<https://www.graphpad.com/quickcalcs/kappa1/>). Inter-test agreement was further evaluated by calculating pairwise Cohen's kappa coefficients and visualized using a lower-triangular matrix heatmap (https://www.bioinformatics.com.cn/plot_basic_matrix_heatmap_064_en) (50). The strength of agreement was assessed using the kappa statistic and interpreted as follows: $\kappa < 0$ indicates no agreement; $\kappa = 0.00-0.20$ indicates slight agreement; $\kappa = 0.21-0.40$ indicates fair agreement; $\kappa = 0.41-0.60$ indicates moderate agreement; $\kappa = 0.61-0.80$ indicates substantial agreement; and $0.81-1.00$ represents perfect agreement (51). Diagnostic concordance among different diagnostics was visualized using Venn diagrams (https://www.bioinformatics.com.cn/plot_basic_proportional_2_or_3_venn_diagram_028_en) (50).

Results

Study population, infection intensity and prevalence

Of these, 270 (49.5%) were female and 275 (50.5%) were male. The mean age for male participants was 26.9 years (95% confidence interval (CI), 24.8–29.0), while the mean age of female participants was 25.7 years (95% CI, 23.6–27.7). Detailed demographic information, including participants' age and sex distribution, is presented in Table S1.

Five diagnostic tests were performed on the clinical samples collected from the study cohort, including the KK method, two copro-DNA-qPCR systems (qPCR1 and qPCR2), and two serological tests (ELISA and LFIA). Based on the KK results, the study cohort was identified as having moderate *S. japonicum* prevalence of 25.1% (95% CI, 21.7–28.9%), with 92.7% of KK-positive individuals exhibiting light infections (Fig. 2A and B). The positivity rate estimates obtained using qPCR1, qPCR2, ELISA and LFIA were 25.9% (95% CI, 22.4–29.7%), 29.9% (95% CI, 26.2–33.9%), 45.3% (95% CI, 41.2–49.5%), and 48.8% (95% CI, 48.8–51.2%), respectively (Fig. 2C and Table S2). Significant differences in positivity rate were observed among the five diagnostic methods, except between the KK and qPCR1 (McNemar's test) (Table S2).

A composite reference as defined in the Methods yielded an overall prevalence estimate of 34.5% (95% CI, 30.6–38.6%) (Fig. 3C). Infection prevalence was significantly higher among males than females according to all the tests (χ^2 , $P < 0.0001$; Fig. 2D, Table S3). Infection intensity based on KK was significantly higher among males than females (Mann-Whitney U test, $P = 0.0240$; Fig. 2E). Participants were further stratified by the KK results. Among KK-positive individuals, the molecular assays and serological assays showed comparable

performance in detecting moderate-to-high-intensity infections (EPG ≥ 100) and light infection intensities with EPG between 10–99 (Fig. 2F). However, differences emerged in the detection of extremely light-intensity infections (EPG 1–9). Among individuals with extremely light infections, 39.3% and 48.2% tested positive by qPCR1 and qPCR2, respectively, whereas 73.2% and 76.8% tested positive by ELISA and LFIA (Fig. 2F). A significant decreasing trend in sensitivity with decreasing infection intensity was observed for qPCR1 ($\chi^2 = 35.15$, $P < 0.001$), qPCR2 ($\chi^2 = 23.19$, $P < 0.001$), and ELISA ($\chi^2 = 6.596$, $P = 0.0102$), but not for LFIA ($\chi^2 = 1.376$, $P = 0.2408$) (chi-square test for trend). Among KK-negative individuals, 11.5% and 16.2% were positive by qPCR1 and qPCR2, respectively. In contrast, relatively higher proportions were positive by the serological assays, with 32.8% testing positive by ELISA and 38.0% by LFIA (Fig. 2F).

Performance based on calibration of composite reference

We next evaluated the performance of each diagnostic method against the composite reference standard. ELISA and LFIA exhibited comparable sensitivities of 84.0%, and 83.0%, respectively. qPCR2 showed a moderate sensitivity of 77.1%, while qPCR1 and KK exhibited relatively lower sensitivities of 72.9% and 70.7%, respectively. In terms of specificity, the KK and copro-DNA-qPCR assays showed high specificity (100%, 97.8% and 95.0% for KK, qPCR1 and qPCR2, respectively) than those of ELISA and LFIA (75.1% and 69.2%, respectively). Among all diagnostic methods, the KK exhibited the highest Youden's J index (0.729), followed by qPCR2 (0.721), whereas LFIA showed the lowest (0.522). The KK and molecular assays demonstrated substantial agreement with the composite reference, while the serological assays showed moderate agreement (Table 1). Inter-test agreement was further evaluated using Cohen's kappa statistic (Fig. 3A). Substantial agreement was observed between qPCR1 and qPCR2 ($\kappa = 0.754$), as well as ELISA and LFIA ($\kappa = 0.798$). Moderate agreement was observed between the KK and qPCR1 ($\kappa = 0.565$), KK and qPCR2 ($\kappa = 0.514$), qPCR1 and ELISA ($\kappa = 0.431$), and qPCR2 and ELISA ($\kappa = 0.443$). Fair agreement was observed between the KK and ELISA ($\kappa = 0.392$), KK and LFIA ($\kappa = 0.328$), and qPCR1 and LFIA ($\kappa = 0.373$) and qPCR2 and LFIA ($\kappa = 0.381$). Focusing on discordant cases between qPCR1 and qPCR2, 16 samples tested positive using qPCR1 but negative by qPCR2, while 38 samples tested positive by qPCR2 but negative by qPCR1 (Table S4). Thus, the proportion of discordant cases is 9.9% (54/545). Among these samples, 13 tested positive by KK, all of which had light infections. There was no difference in Ct value between the two groups of discordant cases (Mann-Whitney U test, $P = 0.2627$). Venn diagram analyses were performed to compare the overlap of positive cases among different combinations of three diagnostic tests that included KK (Fig. 3B). The largest number of shared positive cases ($n = 107$) was identified commonly by the KK, ELISA, and LFIA, whereas the smallest number of shared positive cases ($n = 84$) was identified by the KK, qPCR1, and LFIA.

Table 1
Comparision of diagnostic approaches using a composite reference as the denominator

Diagnostic method	Composite reference		% Sensitivity	% Specificity	% PPV	% NPV	Youden index (J)	Kappa index
	+	-	(95% CI)	(95% CI)	(95% CI)	(95% CI)		
KK	+	137	0	72.9	100	100	87.5	0.729
	-	51	357	(66.1–78.7)	(98.9–100)	(97.3–100)	(83.9–90.4)	(0.722–0.835)
qPCR1	+	133	8	70.7	97.8	94.3	86.4	0.685
	-	55	349	(63.9–76.8)	(95.6–98.9)	(89.2–97.1)	(82.7–89.4)	(0.666–0.790)
qPCR2	+	145	18	77.1	95.0	89.0	88.7	0.721
	-	43	339	(70.6–82.6)	(92.2–96.8)	(83.2–92.9)	(85.2–91.5)	(0.684–0.804)
ELISA	+	158	89	84.0	75.1	64.0	89.9	0.591
	-	30	268	(78.1–88.6)	(70.3–79.3)	(57.8–69.7)	(86.0–92.9)	(0.481–0.620)
LFIA	+	156	110	83.0	69.2	58.6	88.5	0.522
	-	32	247	(77.0–87.7)	(64.2–73.8)	(52.6–64.4)	(84.3–91.8)	(0.404–0.546)

CI Confidence interval, ELISA Enzyme-linked immunosorbent assay, KK Kato-Katz, LFIA Lateral flow immunoassay, NPV negative predictive value, PPV positive predictive value, qPCR Quantitative real-time PCR

Diagnostic performance assessed using LCA

We further assessed performance of the five tests using a two-class LCA model. After accounting for within-class association between ELISA and LFIA, the model separated participants into a Negative profile (74.4%) and a Positive profile (25.6%) (Fig. 4A and Table S5). These class labels describe relative test-result patterns and do not independently confirm infection status. Model simulated sensitivity and specificity estimates for each test are shown in Table 2. The molecular and serological tests had higher sensitivities (all > 90% or close to 90%) than that of KK, whereas KK and the molecular tests had higher specificities (all > 90%) than ELISA and LFIA (Table 2). The molecular tests had highest Youden's J indices, followed by KK and ELISA, whereas LFIA had the lowest Youden's index (Table 2).

Table 2
Diagnostic performance of different tests based on the two-class LCA model

Test	Sensitivity (95% CI) ¹	Specificity (95% CI) ²	Youden index (J)
KK	71.5% (62.9–78.8%)	90.8% (87.5–93.3%)	0.623
qPCR1	92.0% (83.8–96.2%)	96.9% (94.3–98.4%)	0.889
qPCR2	95.4% (88.6–98.2%)	92.7% (89.3–95.1%)	0.881
ELISA	91.0% (84.4–95.0%)	70.5% (65.8–74.8%)	0.615
LFIA	89.4% (82.7–93.8%)	65.3% (60.4–69.9%)	0.547
¹ Corresponds to the observed positive-result probability in Positive profile column of Table S5;			

² Corresponds to the observed negative-result probability in Negative profile column of Table S5.

Within the two-class Negative profile, the probabilities of ELISA- and LFIA-positive results were 29.5% and 34.7%, respectively, suggesting the model did not adequately capture a pattern in which ELISA and LFIA were commonly positive, whereas KK, qPCR1 and qPCR2 were predominantly negative. We therefore fitted a conditionally independent three-class model to examine whether an Intermediate profile was present. The three-class model estimated a prevalence of 52.4% (95% CI, 45.5–59.1%) for the Negative profile, 23.6% (95% CI, 17.8–30.5%) for the Intermediate profile, and 24.1% (95% CI, 20.4–28.2%) for the Positive profile (Fig. 4B and Table S6). These class labels describe relative patterns of test results rather than independently observed diagnoses. In the Negative profile, all five tests were more likely to be negative than positive. In the Intermediate profile, ELISA and LFIA were more likely to be positive, whereas KK, qPCR1 and qPCR2 were more likely to be negative. In the Positive profile, all five tests were more likely to be positive (Fig. 4B).

Comparison of other features for the different diagnostic methods

Beyond diagnostic performance, we compared several key operational characteristics of the five diagnostic tests, including sample requirements, equipment needs, turnaround time, cost, personnel training, and suitability for field deployment (Table 3).

Table 3

Comparison of sample type, equipment requirement, turnaround time, cost and field applicability across the diagnostic tests evaluated in this study.

Test	Sample	Amount	Equipment	Time	Cost [#] (US\$/sample)	Personnel training	Field application
KK	stool	0.05 g / sample (three stool samples, total 6 slides per participant)	Light microscope	30 min ¹ (total 90 min / subject)	0.60 ¹ (total US\$ 1.80 / subject)	Extensive	+
qPCR1	stool	200 mg	Homogeniser; Dry heat block; Centrifuge; Maxwell 16 Instrument; CFX384 Touch Real- Time PCR Detection System; Computer	2 h	6.00 (US\$ 5 for DNA extraction and US\$ 1 for qPCR) ²	Extensive	–
qPCR2	stool	500 mg	Vortex mixer; Dry heat block; Centrifuge; QuantStudio 5 system; Computer	2 h	1.50 (US\$ 0.5 for DNA extraction and US\$ 1 for qPCR) ²	Extensive	–
ELISA	serum	2 µL	Incubator; Microplate reader; Computer	4 h	0.25 (US\$ 5 for one MaxiSorp plate, US\$ 2 for antigen coated on one plate, US\$ 5 for testing reagents and buffer solutions) ²	Extensive	–
LFIA	serum	5 µL	iPhone 8.0; Computer	30 min	2.00	Minimal	+

Not including infrastructure, equipment, labor, and transport cost.

¹Estimated based on duplicate KK thick smear slides;

²Assuming that 48 samples were tested simultaneously on one plate;

ELISA Enzyme-linked immunosorbent assay, KK Kato-Katz, LFIA Lateral flow immunoassay, qPCR Quantitative real-time PCR

Discussion

Schistosomiasis japonica remains a significant public health challenge in the Philippines, with high prevalence rates persisting in several endemic areas (3). Environmental conditions in many endemic regions, including high annual rainfall and extensive freshwater habitats, favor the proliferation of *Oncomelania hupensis quadrasi* snail intermediate hosts and sustain transmission. Furthermore, the zoonotic nature of *S. japonicum*, involving more than 40 mammalian reservoir hosts, complicates control efforts and contributes to ongoing transmission (30, 52). Meanwhile, the implementation of MDA programs has reduced overall infection intensity in many endemic communities. While this represents substantial progress toward disease control, it also presents challenges for accurate diagnosis and effective surveillance.

In this study, we compared the diagnostic performance of the KK technique, molecular detection methods, and serological assays using clinical samples collected from a well-characterized cohort in Alangalang, Leyte Province, Philippines. According to the classification criteria set by the World Health Organization (WHO), this cohort has a moderate prevalence of *S. japonicum* infection, recorded at 25.1% by the KK method, with 92.7% of infected individuals harboring light infections (EPG 1–99). In such settings, it is well known that examination of a single stool sample underestimates the actual prevalence due to the intermittent excretion of schistosome eggs and the small quantity of stool examined using the KK method (17, 53–55). To improve diagnostic sensitivity, duplicate KK slides were prepared from three stool samples collected from each participant. Using the composite reference standard, this approach achieved a moderate sensitivity (72.9%) but absolute specificity. Although molecular diagnostic methods are generally considered more sensitive than parasitological techniques, both qPCR1 and qPCR2 demonstrated diagnostic performance comparable to that of KK, evidenced by the similar Youden's J index. Both serological assays exhibited the highest sensitivity among the five diagnostic tests (83–84%). However, their specificity was mediocre (75.1% and 69.2% for ELISA and LFIA, respectively), suggesting a relatively high proportion of false-positive results. This finding is consistent with a known limitation of AbD-based assays, which may detect antibodies generated by previous infections and therefore cannot reliably distinguish between past and current infections (16, 19). Alternatively, the relatively low specificity may reflect residue infection, recent pre-patent infection/re-infection, or single sex infections, which may be difficult to detect using the KK or copro-DNA based molecular assays. Compared with ELISA, the LFIA showed 6% lower specificity, indicating that further optimization is needed to improve its performance. As a priority for future optimization, the LFIA could be reformulated by replacing Protein G with a more specific monoclonal antibody against human total IgG or IgG isotypes (56, 57). However, this modification would restrict its use to the detection of infections in humans. In addition, optimization of membrane or buffer conditions, adoption of an alternative assay format and use of a strip reader for interpretation may help increase the specificity of the LFIA (20).

High levels of agreement were observed between the ELISA and LFIA assays ($\kappa = 0.798$) and between the two molecular assays ($\kappa = 0.754$). The KK method showed stronger agreement with the molecular assays than with the serological assays, reflecting the fact that both KK and qPCR-based methods detect parasite-derived material associated with active infection. In contrast, agreement between the molecular and serological assays ranged from fair to moderate. This discrepancy is likely attributable to the different biological targets and sample types used by these diagnostic approaches. The copro-DNA qPCR assays detect schistosome egg-derived DNA in stool samples, whereas the serological assays probe host antibodies directed against specific schistosome antigens. The relatively low agreement between molecular and serological methods may be partly explained by the persistence of anti-schistosome antibodies following successful treatment and clearance of parasite eggs from stool samples. Consequently, serological assays may remain positive even in the absence of detectable active egg excretion. Conversely, KK and copro-DNA-based molecular assays may fail to detect some infections because of heterogeneous egg distribution within stool samples, day-to-day variation in egg excretion, or infections involving unisexual, senescent, or low-fecundity worms that produce few or no detectable eggs (58, 59).

LCA has been widely used to evaluate diagnostic performance in the absence of a gold standard test (60–62). In this study, the two-class LCA model provided model-based performance for the five diagnostic assays. Compared with the results obtained using the composite-reference, the specificity of KK decreased by approximately 10%, whereas the sensitivities of the molecular assays increased by roughly 23–30% while maintaining high specificity. The sensitivities of the serological assays also increased about 8%, accompanied by an approximately 6% decrease in specificity. Youden's J indices for the molecular approaches increased about 0.16–0.2 under the two-class LCA model. Notably, qPCR2 had a relatively high Youden's index under both two-class LCA and the composite-reference approach. Similarly, a previous LCA-based assessment of the performance of 11 diagnostic tests reported comparable performance patterns for copro-DNA-based PCR and a commercial ELISA (42). Overall, both the two-class LCA model and composite-reference approach indicated high specificity for the KK and molecular approaches, and high sensitivity and moderate specificity for serological tests.

Two-class models, typically based on distinguishing infected from non-infected status, have predominantly been used when applying LCA to evaluate diagnostic performance of assays targeting parasite eggs, parasite-derived antigens, or parasite-originated nucleic acids (62–65). In contrast, this study evaluated serological assays based on the detection of host antibodies alongside KK and qPCR assays. Thus, an additional three-class model was fitted to distinguish an Intermediate profile from the two-class Negative profile. The Intermediate profile, characterized by predominantly positive serological test results and predominantly negative KK/qPCR results, may represent previous infection/exposure, single-sex worm infection, pre-patent infection/re-infection or persistent very-low-intensity infection, like residue infection following MDA. The identification of this intermediate profile may help explain the moderate specificities of ELISA and LFIA observed in both the two-class LCA model and the composite reference analysis.

We further compared practical considerations relevant to the development and implementation of diagnostic tools for *S. japonicum* infection, with particular emphasis on operational feasibility, such as equipment and personnel training requirements, turnaround time, cost, and field applicability. Among the five diagnostic methods evaluated, the KK technique required examination of three stool samples per participant to achieve

the diagnostic performance reported in this study, whereas both molecular assays achieved comparable or superior performance using a single stool sample. Serological assays offer additional practical advantages by avoiding the inherent heterogeneity and unpleasant odor associated with stool samples and requiring only a small volume of serum. Consistent with these considerations, barangay health workers preferred serum to stool samples in a sample acceptability survey (data not shown). Additionally, plasma isolated from finger prick blood can also be used for serological assays, potentially improving participant compliance and facilitating large-scale sample collection in endemic communities (22). Turnaround time also differed substantially among the diagnostic methods. LFIA required about 30 min to generate results, compared with approximately 90 min for examination of six KK thick-smear slides from a single individual, and 2 and 4 h for the molecular assays and ELISA, respectively. These estimates do not include the additional time required to collect three stool samples over a one-week period for the KK analysis. Cost is another important consideration in the development of diagnostic tools for schistosomiasis, a neglected tropical disease that disproportionately affects individuals in low- and middle-income countries (LMICs). Based on consumable and reagent costs, the estimated cost per participant in this study was approximately US\$ 1.80, 6.00, 1.50, 0.25, and 2.00 for the KK, qPCR1, qPCR2, ELISA, and LFIA, respectively. The relatively high cost of qPCR1 was largely attributable to the use of the Maxwell kit for DNA extraction (45). In contrast, qPCR2, which incorporated a cost-effective NaOH-based DNA extraction method, reduced the overall assay cost to below that of LFIA while maintaining strong diagnostic performance (45). Nevertheless, it should be noted that estimated costs for molecular and serological assays were based on a batch testing of 48 samples. Testing a smaller number of samples simultaneously would substantially increase the per-sample costs of qPCR1, qPCR2, and ELISA. Thus, these methods are more economically suited to high-throughput testing, where batch processing improves efficiency and reduces per-sample costs. Despite their relatively high diagnostic accuracy, molecular assays require specialized laboratory equipment and trained personnel, which limit their applicability in resource-constrained field settings (11, 66). Nevertheless, significant advances have been made in facilitating molecular assays for POC diagnosis of infectious diseases (11, 67, 68). The qPCR2 workflow could potentially be transformed into a field-friendly POC test by integrating portable centrifuging and qPCR machines (45, 66). In contrast, both KK and LFIA offer practical advantages in terms of field deployment. KK requires minimal equipment and low consumable costs, whereas LFIA combines rapid turnaround time, ease of use, minimal equipment requirements, and high sensitivity, making it potentially useful for community-based surveillance and screening programs (20–22).

A key strength of the study is the use of an intensified KK sampling protocol involving three stool samples with duplicate slides. The resulting sensitivity of 70.6% for KK against the composite reference, however, came at a considerable operational cost, requiring three stool samples collected over one week, preparation of six slides for each participant, and approximately 90 min of microscopy per individual testing (Table 3). Such an intensive protocol may be less feasible for routine surveillance or large-scale MDA monitoring, as the single-stool, single-slide KK approach is commonly used in routine programmes, with an expected reduction in sensitivity. There are limitations in the study. First, the composite reference cannot be considered as a gold standard. To lower the impact of potential detection of past infection of serological assays, the weight of serological assays in the composite reference was limited. Such approach may introduce incorporation bias and potentially underestimate the diagnostic performance of serological assays. Second, the infection prevalence observed in males was significantly higher than females. However, no data on occupational activities, water-contact

behaviors, or other potential exposure factors has been collected, precluding assessment of whether differences in these factors could explain the observed sex disparity.

Conclusions

This study compared the diagnostic performance and operational characteristics of five methods for detecting *S. japonicum* infection in a moderately endemic setting in the Philippines, where most KK-positive individuals exhibited light-intensity infections. The findings highlighted the strengths and limitations of each diagnostic approach. The intensified KK method used in this study demonstrated high specificity and a moderate sensitivity, but at a considerable operational cost. Based on the composite-reference approach, the serological assays achieved the highest sensitivity but had moderate specificity. The two-class LCA model provided complementary, model-based estimates of diagnostic performance, with the molecular assays demonstrating high sensitivity and specificity. In addition, the three-class model further identified an Intermediate profile, highlighting potential heterogeneity of *S. japonicum* infection and exposure patterns that may help explain the moderate specificity of the serological assays. Considering both operational characteristics and diagnostic performance, LFIA represents a useful tool for rapid field-based screening, whereas the molecular detection system incorporating the NaOH-based DNA extraction (qPCR2) combined relatively high diagnostic performance with a lower assay cost. Further adaptation of qPCR2 into a field-deployable format, together with evaluation across different epidemiological and programmatic settings, will help to determine its potential utility for schistosomiasis surveillance and control.

Abbreviations

AIC

Akaike Information Criterion

AUC

Area under the ROC curve

BIC

Bayesian Information Criterion

CI

Confidence interval

DALYs

disability-adjusted life years

ddPCR

droplet digital PCR

ELISA

Enzyme-Linked Immunosorbent Assay

EPG

Eggs per gram of faeces

IHA

Indirect Hemagglutination Assay

KK

Kato-Katz

LAMP

Loop-mediated isothermal amplification

LCA

Latent class analysis

LFAs

Lateral flow assays

LFIA

Lateral flow immunoassay

LMICs

Low- and middle-income countries

MDA

mass drug administration

NPV

Negative predictive value

POC-CCA

Point-of-care circulating cathodic antigen

PPV

Positive predictive value

qPCR

Quantitative real-time PCR

UCP-LF-CAA

up-converting phosphor-lateral flow circulating anodic antigen

ROC

Receiver operating characteristic

RPA

recombinase polymerase amplification

WHO

World Health Organization.

Declarations

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Authors' contributions

CAG and PC formulated the research aims and prepared the study protocol. CAG and PC supervised the research. CAG, MAJ, EA, MI, and PC provided study materials. EJT, YM, CAG, MAJ, EA, MI, YS, MOS, MS, DJG, LJ, MLM, and PC developed the methodology, and collected and analysed the data. PC drafted the original manuscript. EJT, YM, CAG, MAJ, EA, MI, YS, MOS, MS, DJG, LJ, MLM, and PC critically reviewed and edited the manuscript. The authors read and approved the final version of the manuscript.

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Data availability statement

All data supporting the findings of this study are available within the paper and Supplementary data.

Ethics approval and consent to participate

Human ethics approval for this work was obtained from the QIMRB Human Ethics Committees (P3797) and the Institutional Review Board of the Research Institute for Tropical Medicine (RITM) (ID No. 2022–16), Muntinlupa, Philippines. Written informed consent was obtained from all participants aged 13 years or older. For individuals aged 12 years or younger, informed consent was obtained from their parents or legal guardians. The study was conducted in accordance with the National Statement on Ethical Conduct in Human Research (July 2018).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. WHO (2023) Schistosomiasis. World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>. Accessed: 21 Dec 2025
2. McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ (2018) Zhou X-N. Schistosomiasis. *Nat Rev Dis Primers* 4(1):13
3. Tabilin EJ, Gray DJ, Jiz MA, Mationg ML, Inobaya M, Avenido-Cervantes E et al (2025) Schistosomiasis in the Philippines: A Comprehensive Review of Epidemiology and Current Control. *Trop Med Infect Dis.* ;10(2)
4. Olveda RM, Gray DJ (2019) Schistosomiasis in the Philippines: Innovative Control Approach is Needed if Elimination is the Goal. *Trop Med Infect Dis* 4(2):66
5. Ezeamama AE, Bustinduy AL, Nkwata AK, Martinez L, Pabalan N, Boivin MJ et al (2018) Cognitive deficits and educational loss in children with schistosome infection-A systematic review and meta-analysis. *PLoS Negl Trop Dis* 12(1):e0005524
6. Tamarozzi F, Fittipaldo VA, Orth HM, Richter J, Buonfrate D, Riccardi N et al (2021) Diagnosis and clinical management of hepatosplenic schistosomiasis: A scoping review of the literature. *PLoS Negl Trop Dis* 15(3):e0009191

7. Cai P, Gobert GN, You H, McManus DP (2016) The Tao survivorship of schistosomes: implications for schistosomiasis control. *Int J Parasitol* 46(7):453–463
8. Abeleda AI, Lucero-Prisno DEI, Ogaya JB (2024) Addressing schistosomiasis in the Philippines: Need for a coordinated intersectoral effort. *Asian Pac J Trop Med* 17(11):479–480
9. Revolteado MJ, Sato MO, Valencia J, Jiz M, Cervantes E, Aniceto R et al (2025) Seasonality and dynamics of schistosomiasis in the environment: usefulness of environmental DNA (eDNA) surveillance system at a community level for risk mapping schistosomiasis in Ekiran Village. *Leyte Philippines mSphere* 10(4):e0106124
10. Valencia JE, Sato MO, Revolteado MJ, Prasayasith P, Jiz M, Cervantes EA et al (2025) Soil-based environmental DNA enables detection of *Oncomelania hupensis quadrasi* and *Schistosoma japonicum* microhabitats for schistosomiasis japonica surveillance and control in the Philippines. *Infect Dis Poverty* 14(1):110
11. Ally O, Kanoi BN, Ochola L, Nyanjom SG, Shiluli C, Misinzo G et al (2024) Schistosomiasis diagnosis: Challenges and opportunities for elimination. *PLoS Negl Trop Dis* 18(7):e0012282
12. Weerakoon KG, Gobert GN, Cai P, McManus DP (2015) Advances in the Diagnosis of Human Schistosomiasis. *Clin Microbiol Rev* 28(4):939–967
13. Cavalcanti MG, Silva LF, Peralta RH, Barreto MG, Peralta JM (2013) Schistosomiasis in areas of low endemicity: a new era in diagnosis. *Trends Parasitol* 29(2):75–82
14. Mesquita SG, Caldeira RL, Favre TC, Massara CL, Beck L, Simões TC et al (2022) Assessment of the accuracy of 11 different diagnostic tests for the detection of Schistosomiasis mansoni in individuals from a Brazilian area of low endemicity using latent class analysis. *Front Microbiol* 13:1048457
15. Xu X, Zhang Y, Lin D, Zhang J, Xu J, Liu YM et al (2014) Serodiagnosis of *Schistosoma japonicum* infection: genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. *Lancet Infect Dis* 14(6):489–497
16. Cai P, Weerakoon KG, Mu Y, Olveda RM, Ross AG, Olveda DU et al (2019) Comparison of Kato Katz, antibody-based ELISA and droplet digital PCR diagnosis of schistosomiasis japonica: Lessons learnt from a setting of low infection intensity. *PLoS Negl Trop Dis* 13(3):e0007228
17. Cai P, Weerakoon KG, Mu Y, Olveda DU, Piao X, Liu S et al (2017) A Parallel Comparison of Antigen Candidates for Development of an Optimized Serological Diagnosis of Schistosomiasis Japonica in the Philippines. *EBioMedicine* 24:237–246
18. Mu Y, Gordon CA, Olveda RM, Ross AG, Olveda DU, Marsh JM et al (2022) Identification of a linear B-cell epitope on the *Schistosoma japonicum* saposin protein, SjSAP4: Potential as a component of a multi-epitope diagnostic assay. *PLoS Negl Trop Dis* 16(7):e0010619
19. Hinz R, Schwarz NG, Hahn A, Frickmann H (2017) Serological approaches for the diagnosis of schistosomiasis – A review. *Mol Cell Probes* 31:2–21
20. Tabilin EJ, Gordon CA, Mu Y, Jiz M, Inobaya M, Avenido-Cervantes E et al (2024) Development of a latex microsphere-based lateral flow immunoassay for the diagnosis of schistosomiasis japonica. *PLoS Negl Trop Dis* 18(12):e0012742
21. Mu Y, Rivera J, McManus DP, Weerakoon KG, Ross AG, Olveda RM et al (2023) Comparative assessment of the SjSAP4-incorporated gold immunochromatographic assay for the diagnosis of human schistosomiasis

japonica. Front Public Health 11:1249637

22. Mu Y, McManus DP, Gordon CA, You H, Ross AG, Olveda RM et al (2023) Development and assessment of a novel gold immunochromatographic assay for the diagnosis of schistosomiasis japonica. Front Immunol 14:1165480
23. Cavalcanti MG, Cunha AFA, Peralta JM (2019) The Advances in Molecular and New Point-of-Care (POC) Diagnosis of Schistosomiasis Pre- and Post-praziquantel Use: In the Pursuit of More Reliable Approaches for Low Endemic and Non-endemic Areas. Front Immunol 10:858
24. Peralta JM, Cavalcanti MG (2018) Is POC-CCA a truly reliable test for schistosomiasis diagnosis in low endemic areas? The trace results controversy. PLoS Negl Trop Dis 12(11):e0006813
25. Graeff-Teixeira C, Favero V, Pascoal VF, de Souza RP, Rigo FV, Agnese LHD et al (2021) Low specificity of point-of-care circulating cathodic antigen (POCCCA) diagnostic test in a non-endemic area for schistosomiasis mansoni in Brazil. Acta Trop 217:105863
26. Sousa MS, van Dam GJ, Pinheiro MCC, de Dood CJ, Peralta JM, Peralta RHS et al (2019) Performance of an Ultra-Sensitive Assay Targeting the Circulating Anodic Antigen (CAA) for Detection of *Schistosoma mansoni* Infection in a Low Endemic Area in Brazil. Front Immunol 10:682
27. Hamway Y, Josten T, Abdellatif S, Mukherjee P, Schluckebier J, Berry SPD et al (2026) Diagnostic accuracy of plasma cell-free DNA qPCR for *Schistosoma haematobium* assessed by Bayesian latent class analysis in a cohort of pregnant women from Lambaréné, Gabon. Infect Dis Poverty. ;15(1)
28. Fasogbon IV, Ondari EN, Tusubira D, Ogbonnia EC, Ashley J, Sasikumar S et al (2024) A critical review of the limitations of current diagnostic techniques for schistosomiasis. All Life 17(1):2379305
29. Halili S, Grant JR, Pilotte N, Gordon CA, Williams SA (2021) Development of a novel real-time polymerase chain reaction assay for the sensitive detection of *Schistosoma japonicum* in human stool. PLoS Negl Trop Dis 15(10):e0009877
30. Gordon CA, Acosta LP, Gobert GN, Jiz M, Olveda RM, Ross AG et al (2015) High prevalence of *Schistosoma japonicum* and *Fasciola gigantica* in bovines from Northern Samar, the Philippines. PLoS Negl Trop Dis 9(2):e0003108
31. Weerakoon KG, Gordon CA, Williams GM, Cai P, Gobert GN, Olveda RM et al (2017) Droplet Digital PCR Diagnosis of Human Schistosomiasis: Parasite Cell-Free DNA Detection in Diverse Clinical Samples. J Infect Dis 216(12):1611–1622
32. Weerakoon KG, Gordon CA, Gobert GN, Cai P, McManus DP (2016) Optimisation of a droplet digital PCR assay for the diagnosis of *Schistosoma japonicum* infection: A duplex approach with DNA binding dye chemistry. J Microbiol Methods 125:19–27
33. Aula OP, McManus DP, Jones MK, You H, Cai P, Gordon CA (2023) Optimisation of the DNA dipstick as a rapid extraction method for *Schistosoma japonicum* in infected mice samples and spiked human clinical samples. Infect Dis Poverty 12(1):71
34. Qin Z-Q, Xu J, Feng T, Lv S, Qian Y-J, Zhang L-J et al (2018) Field Evaluation of a Loop-Mediated Isothermal Amplification (LAMP) Platform for the Detection of *Schistosoma japonicum* Infection in *Oncomelania hupensis* Snails. Trop Med Infect Dis 3(4):124
35. Belizario VY, delos Trinos JPCR, Sison OT, Destura RV, Medina JR, Gigataras AJE et al (2022) Evaluation of loop-mediated isothermal amplification assay and enzyme-linked immunosorbent assay in detecting

Schistosoma japonicum in Siargao Island, Surigao del Norte, the Philippines. Acta Trop 228:106306

36. Mesquita SG, Donnelly O, Archer J, Lugli EB, Webster B (2025) Advances in the recombinase polymerase amplification platform for urogenital schistosomiasis diagnosis (ShDral-RPA) towards implementation at the point-of-care. Sci Rep 15(1):13590
37. Mesquita SG, Gadd G, Coelho FS, Cieplinski A, Emery A, Lugli EB et al (2024) Laboratory and field validation of the recombinase polymerase amplification assay targeting the *Schistosoma mansoni* mitochondrial minisatellite region (SmMIT-RPA) for snail xenomonitoring for schistosomiasis. Int J Parasitol 54(5):247–256
38. Deng W, Wang S, Wang L, Lv C, Li Y, Feng T et al (2022) Laboratory Evaluation of a Basic Recombinase Polymerase Amplification (RPA) Assay for Early Detection of *Schistosoma japonicum*. Pathogens. ;11(3)
39. Sun K, Xing W, Yu X, Fu W, Wang Y, Zou M et al (2016) Recombinase polymerase amplification combined with a lateral flow dipstick for rapid and visual detection of *Schistosoma japonicum*. Parasit Vectors 9(1):476
40. Xing W, Yu X, Feng J, Sun K, Fu W, Wang Y et al (2017) Field evaluation of a recombinase polymerase amplification assay for the diagnosis of *Schistosoma japonicum* infection in Hunan province of China. BMC Infect Dis 17(1):164
41. Tabios IKB, Sato MO, Tantengco OAG, Fornillos RJC, Kirinoki M, Sato M et al (2022) Diagnostic Performance of Parasitological, Immunological, Molecular, and Ultrasonographic Tests in Diagnosing Intestinal Schistosomiasis in Fieldworkers From Endemic Municipalities in the Philippines. Front Immunol 13:1048457
42. Mesquita SG, Caldeira RL, Favre TC, Massara CL, Beck LCNH, Simões TC et al (2022) Assessment of the accuracy of 11 different diagnostic tests for the detection of Schistosomiasis mansoni in individuals from a Brazilian area of low endemicity using latent class analysis. Front Microbiol 13:1048457
43. Fuss A, Mazigo HD, Tappe D, Kasang C, Mueller A (2018) Comparison of sensitivity and specificity of three diagnostic tests to detect *Schistosoma mansoni* infections in school children in Mwanza region, Tanzania. PLoS ONE 13(8):e0202499
44. Vonghachack Y, Sayasone S, Khieu V, Bergquist R, van Dam GJ, Hoekstra PT et al (2017) Comparison of novel and standard diagnostic tools for the detection of *Schistosoma mekongi* infection in Lao People's Democratic Republic and Cambodia. Infect Dis Poverty 6(1):127
45. Tabilin EJ, Gordon CA, Jiz MA, Avenido-Cervantes E, Inobaya M, Sato MO et al (2026) Comparison of Maxwell Kit-Based and NaOH-Based DNA Extraction Incorporated copro-DNA-SjTR1-qPCR Systems: Insights from Detecting Low-Intensity *Schistosoma Japonicum* Infections. Mol Diagn Ther. 10.1007/s40291-026-00883-0
46. Collins J, Huynh M (2014) Estimation of diagnostic test accuracy without full verification: a review of latent class methods. Stat Med 33(24):4141–4169
47. HAGENAARS JA (1988) Latent Structure Models with Direct Effects between Indicators:Local Dependence Models. Sociol Methods Res 16(3):379–405
48. Galindo Garre F, Vermunt JK (2006) Avoiding Boundary Estimates in Latent Class Analysis by Bayesian Posterior Mode Estimation. Behaviormetrika 33(1):43–59

49. Linzer DA, Lewis JB (2011) poLCA: An R Package for Polytomous Variable Latent Class Analysis. *J Stat Softw* 42(10):1–29
50. Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G et al (2023) SRplot: A free online platform for data visualization and graphing. *PLoS ONE* 18(11):e0294236
51. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* 33(1):159–174
52. Jiz M, Mingala C, Fu ZQ, Adriatico M, Lu K, Jarilla B et al (2021) High prevalence of *Schistosoma japonicum* by perfusion in naturally exposed water buffalo in a region of the Philippines endemic for human schistosomiasis. *PLoS Negl Trop Dis* 15(9):e0009796
53. Oliveira WJ, Magalhaes FDC, Elias AMS, de Castro VN, Favero V, Lindholz CG et al (2018) Evaluation of diagnostic methods for the detection of intestinal schistosomiasis in endemic areas with low parasite loads: Saline gradient, Helminthex, Kato-Katz and rapid urine test. *PLoS Negl Trop Dis* 12(2):e0006232
54. Lamberton PH, Kabatereine NB, Oguttu DW, Fenwick A, Webster JP (2014) Sensitivity and specificity of multiple Kato-Katz thick smears and a circulating cathodic antigen test for *Schistosoma mansoni* diagnosis pre- and post-repeated-praziquantel treatment. *PLoS Negl Trop Dis* 8(9):e3139
55. Colley DG, Andros TS, Campbell CH (2017) Jr. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs? *Infect Dis Poverty* 6(1):63
56. Pearson MS, Tedla BA, Mekonnen GG, Proietti C, Becker L, Nakajima R et al (2021) Immunomics-guided discovery of serum and urine antibodies for diagnosing urogenital schistosomiasis: a biomarker identification study. *Lancet Microbe* 2(11):e617–e26
57. Sadaow L, Rodpai R, Smout MJ, Nakajima R, Boonroumkaew P, Sotillo J et al (2025) Immunomics-guided biomarker discovery for human liver fluke infection and infection-associated cholangiocarcinoma. *Nat Commun* 16(1):5965
58. Alemu G, Nibret E, Munshea A, Anegagrie M, Flores-Chávez M, Hailu T et al (2025) Comparative performance of Kato-Katz, POC-CCA and real-time PCR in detecting *Schistosoma mansoni* infection at different endemicity settings in northwest Ethiopia: a cross-sectional study. *Trop Med Health* 53(1):103
59. Okoyo C, Simiyu E, Njenga SM, Mwandawiro C (2018) Comparing the performance of circulating cathodic antigen and Kato-Katz techniques in evaluating *Schistosoma mansoni* infection in areas with low prevalence in selected counties of Kenya: a cross-sectional study. *BMC Public Health* 18(1):478
60. Moon M, Wu HW, Jiz M, Maldonado S, Kurtis JD, Friedman JF et al (2024) Evaluation of sensitivity and specificity of Kato-Katz and circulating cathodic antigen in terms of *Schistosoma japonicum* using latent class analysis. *Sci Rep* 14(1):8164
61. Clements MN, Corstjens P, Binder S, Campbell CH Jr., de Dood CJ, Fenwick A et al (2018) Latent class analysis to evaluate performance of point-of-care CCA for low-intensity *Schistosoma mansoni* infections in Burundi. *Parasit Vectors* 11(1):111
62. Hamway Y, Josten T, Abdellatif S, Mukherjee P, Schluckebier J, Berry SPD et al (2026) Diagnostic accuracy of plasma cell-free DNA qPCR for *Schistosoma haematobium* assessed by Bayesian latent class analysis in a cohort of pregnant women from Lambarene, Gabon. *Infect Dis Poverty* 15(1):50

63. Calvo-Urbano B, Leger E, Gabain I, De Dood CJ, Diouf ND, Borlase A et al (2023) Sensitivity and specificity of human point-of-care circulating cathodic antigen (POC-CCA) test in African livestock for rapid diagnosis of schistosomiasis: A Bayesian latent class analysis. *PLoS Negl Trop Dis* 17(5):e0010739
64. Lorenz E, Razafindrakoto R, Rausche P, Rasolojaona ZT, Razafindralava NM, Zerbo A et al (2025) Detecting *Schistosoma* infections in endemic countries: a diagnostic accuracy study in rural Madagascar. *Infect Dis Poverty* 14(1):20
65. Ngwewondo A, Mbah CE, Sama KO, Wepnje GB, Livo FE, Monya E et al (2026) Molecular, parasitological and clinical diagnosis of female genital schistosomiasis: A diagnostic yield and comparative accuracy study using latent class analysis. *Parasitol Res* 125(1):111
66. Mu Y, Egwang TG, Rowel C, Adriko M, Jones MK, Pangaribuan HU et al (2026) Comparative evaluation of *Sm1-7*-qPCR systems incorporated with Maxwell kit-based and NaOH-based DNA extraction methods for the detection of *Schistosoma mansoni* infection. *Infect Dis Poverty* 15(1):54
67. Rivera J, Mu Y, Gordon CA, Jones MK, Cheng G, Cai P (2024) Current and upcoming point-of-care diagnostics for schistosomiasis. *Trends Parasitol* 40(1):60–74
68. Gavina K, Franco LC, Khan H, Lavik J-P, Relich RF (2023) Molecular point-of-care devices for the diagnosis of infectious diseases in resource-limited settings – A review of the current landscape, technical challenges, and clinical impact. *J Clin Virol* 169:105613

Figures

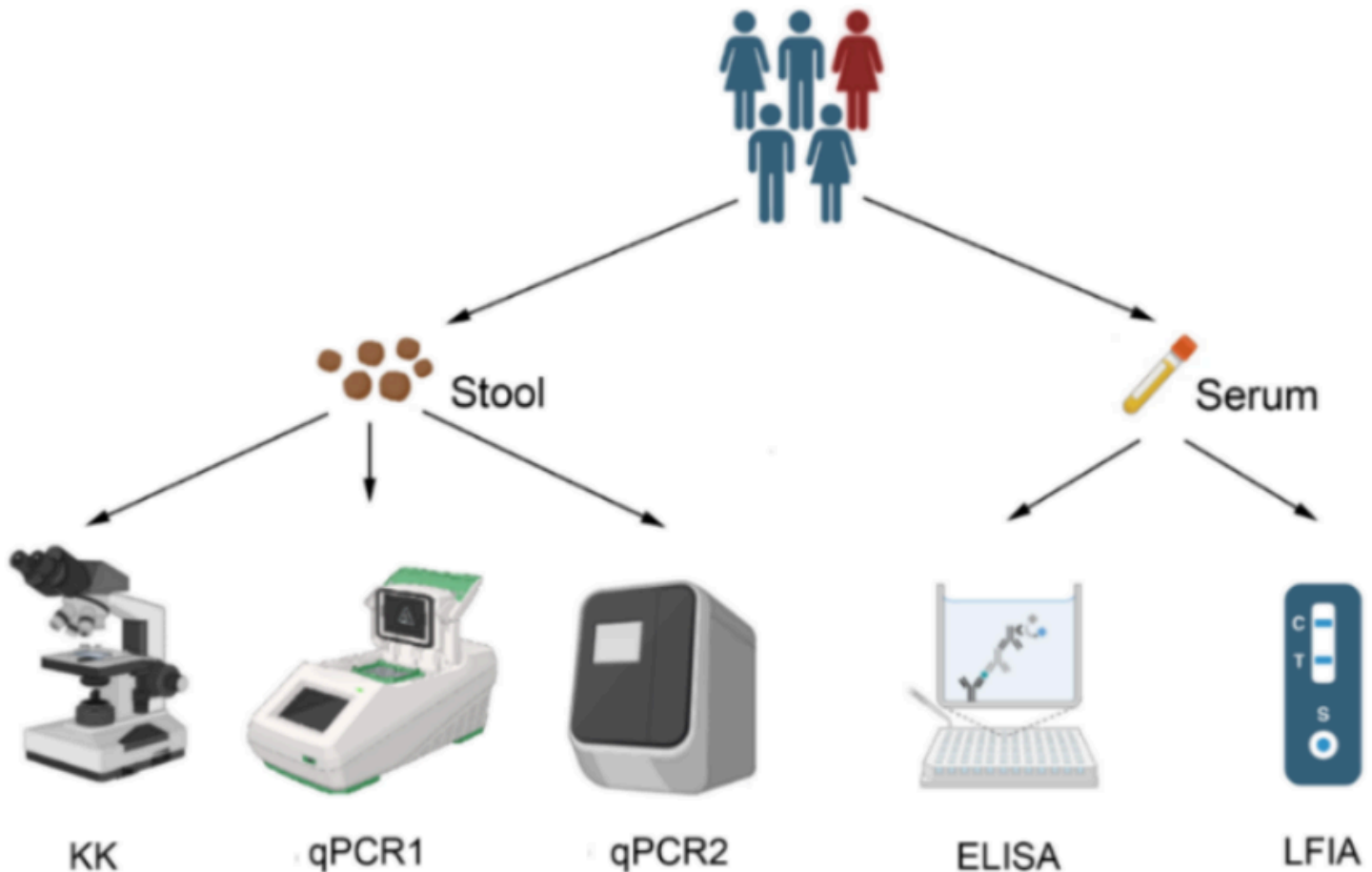


Figure 1

Schematic overview of the study design and diagnostic methods evaluated. Clinical samples were collected from participants residing in Ekiran, Alangalang, Leyte Province, the Philippines, a village endemic for schistosomiasis japonica. For each participant, three stool samples were collected, and each sample was examined using duplicate Kato–Katz (KK) slides. Molecular diagnostics were performed on the stool samples ($n = 554$) collected on the first day using two copro-DNA-qPCR assays, *SjTR1*-qPCR-S1 and *SjTR1*-qPCR-S2 (designated as qPCR1 and qPCR2, respectively) (45). Serum samples ($n= 549$) were tested using the SjSAP4-ELISA and the SjSAP4-incorporated LFIA (20). Based on the availability of results for all five tests, data from 545 participants were included in the present comparative study. Figure created with BioRender. *ELISA* Enzyme-linked immunosorbent assay, *EPG* eggs per gram of faeces, *KK* Kato-Katz, *LFIA* Lateral flow immunoassay, *qPCR* Quantitative real-time PCR

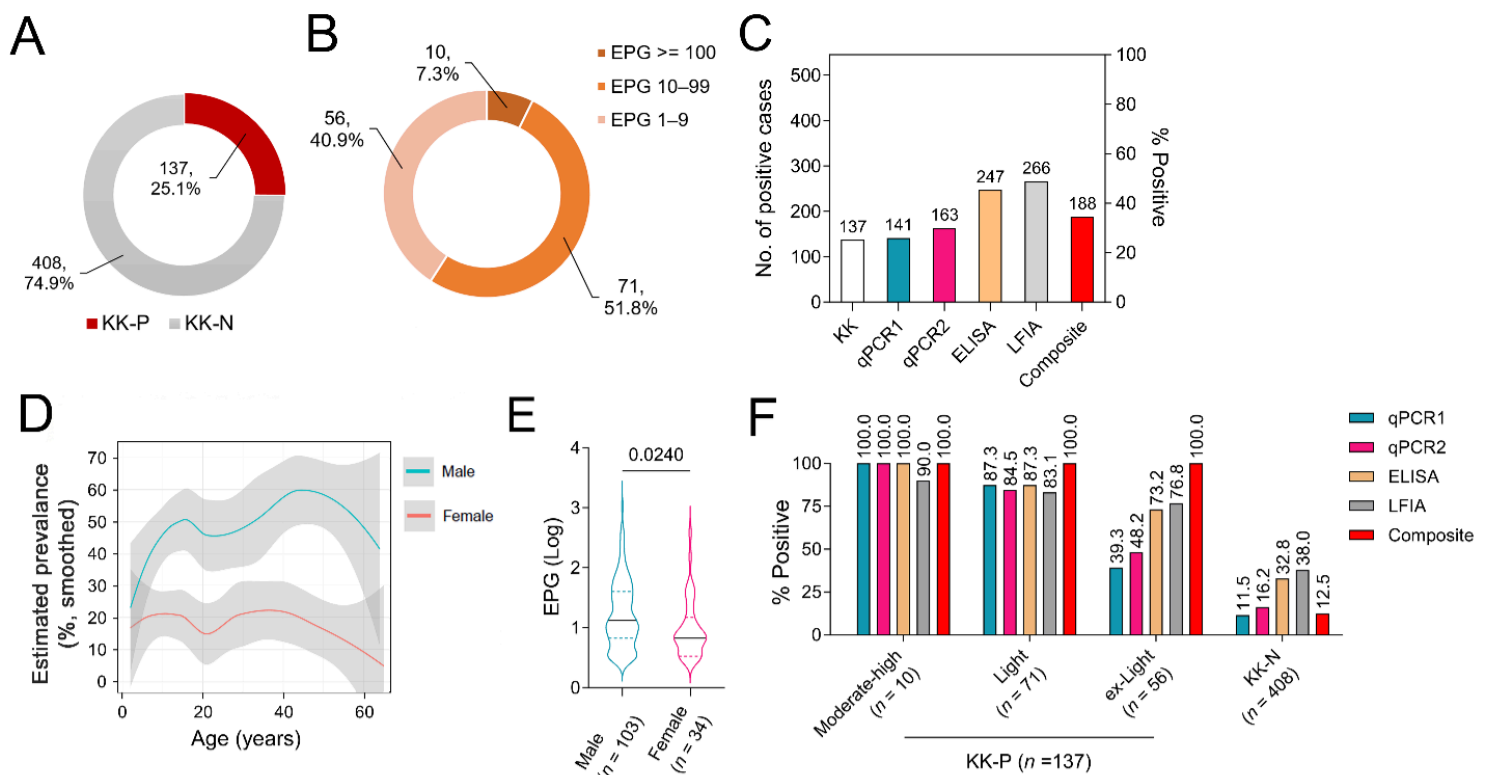


Figure 2

Comparison of diagnostic tests for *S. japonicum* infection. **A)** Number and percentage of KK positive and negative individuals of the study cohort. **B)** Number and percentage of KK-positive individuals with different infection intensities. **C)** Positivity rates determined by the different diagnostic methods. **D)** Age smoothing curves of *S. japonicum* prevalence stratified by sex, based on the composite reference. The shaded area around each curve represents 95% CI. The age range was 2–64 years for males and 2–65 years for females. **E)** Comparison of *S. japonicum* infection intensity, determined by the KK method, between male and female participants (Mann-Whitney *U* test, $P = 0.0240$). **F)** Positivity rates determined by different tests stratified by *S. japonicum* infection status and intensity. *ELISA* Enzyme-linked immunosorbent assay, *EPG* eggs per gram of faeces, *KK* Kato-Katz, *KK-P* Kato-Katz positive, *KK-N* Kato-Katz negative, *LFIA* Lateral flow immunoassay, *qPCR* Quantitative real-time PCR

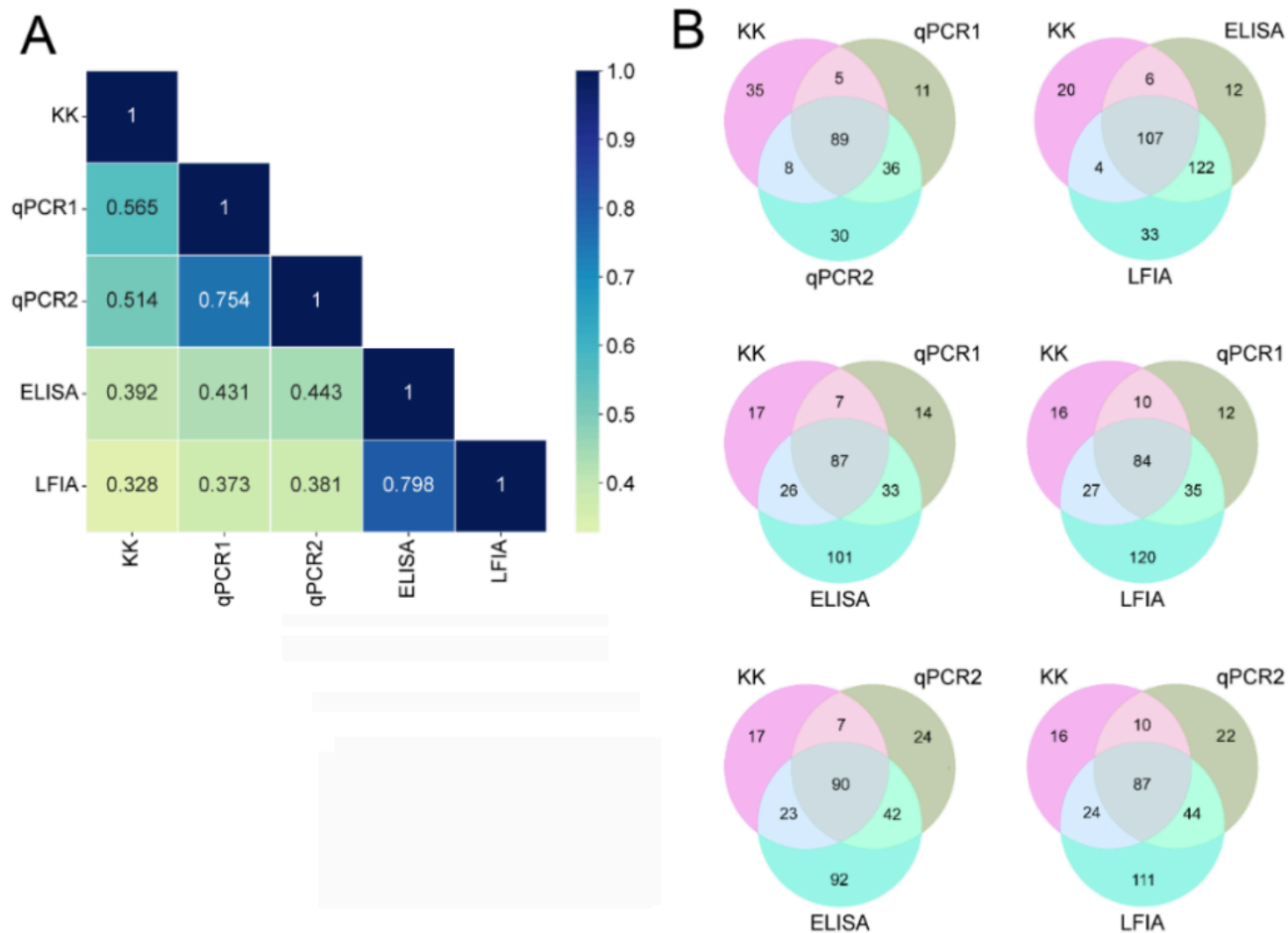


Figure 3

Inter-test agreement analysis. A) Agreement matrices between each test analyzed as the total cohort ($n = 545$). Kappa values were visualized with correlation in a lower triangle heatmap. **B)** Venn diagrams of all positive cases per diagnostic method for three different tests that include the KK. *ELISA* Enzyme-linked immunosorbent assay, *KK* Kato-Katz, *LFIA* Lateral flow immunoassay, *qPCR* Quantitative real-time PCR

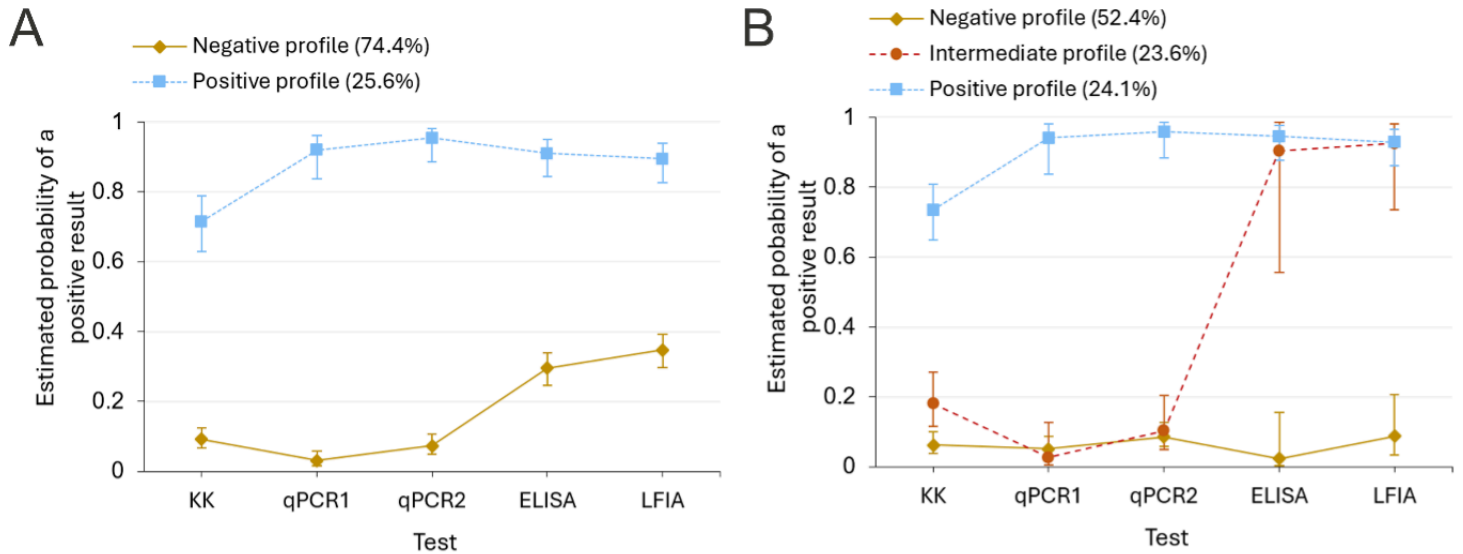


Figure 4

Estimated latent class profiles based on two- and three-class LCA models. A) Two-class LCA model: Class 1, Negative profile; Class 2, Positive profile. B). Three-class LCA model: Class 1, Negative profile; Class 2, Intermediate profile; Class 3, Positive profile. Percentages in the legend indicate the estimated prevalence of each latent class. Points show the estimated probability of a positive test result for each test within each class. Error bars represent approximately 95% CIs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.xlsx](#)