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Real-time PCR and LAMP assays for the diagnosis of *Schistosoma mansoni* using urine samples: comparison of two DNA extraction methods

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Abstract

Currently, molecular tests on urine samples are being developed as complementary diagnostic tools for *Schistosoma mansoni* alongside parasitological methods. This study aimed to evaluate urine-based real-time PCR and LAMP using DNA extracted either with a commercial kit or by phenol–chloroform for detecting *S. mansoni*. Following ethical approval, stool samples were collected from 200 schoolchildren and examined using the Kato-Katz technique. A subset of 25 urine samples (20 *S. mansoni* positive and 5 negative by Kato-Katz) was tested by both real-time PCR and LAMP using the two DNA extraction methods. Kato-Katz diagnosed 36 positive cases, corresponding to a prevalence of 18%. Among them, 38.9%, 55.6% and 5.6% had light, moderate and heavy infections. The urine-based real-time PCR and LAMP commercial extraction kit detected all Kato–Katz positive cases, including those with low infection intensity. Moreover, real-time PCR and LAMP identified 4 and 2 cases, respectively, among Kato–Katz negatives. In contrast, real-time PCR and LAMP phenol/chloroform missed 2 and 3 Kato–Katz positives respectively, each of low infection intensity and LAMP detected one additional case negative by Kato–Katz. The highest sensitivity was observed in real-time PCR (100%) and the LAMP (92%) using the commercial kit, followed by Kato-Katz (83%) and the LAMP phenol/chloroform (75%). **Conclusion** Urine samples are an increasingly validated for *S. mansoni* diagnosis. The commercial kit outperformed phenol/chloroform for *S. mansoni* DNA extraction. Real-time PCR showed the highest performance and LAMP demonstrated a capability as a sensitive, specific, and rapid test suited for resource-limited settings. Although phenol/chloroform remains a cost-effective option, it requires further optimization.

Keywords: *Schistosoma mansoni,* Kato-Katz, urine samples, real-time PCR, LAMP, commercial DNA extraction, phenol/chloroform.

Introduction

Schistosomiasis remains a persistent public health challenge in tropical and subtropical regions. It affects the human population, particularly children, who are higher-risk group in endemic areas (Aliyi et al., 2024). Schistosomiasis is ranked second after malaria in terms of morbidity and mortality, 800 million human beings are at risk and around 200,000 may die each year from the disease (Sady etal., 2015, Aliyi et al., 2024). *Schistosoma mansoni* (*S. mansoni*) is the most prevalent and common cause of intestinal and hepatic schistosomiasis in Sub-Saharan, and South America (Frahm et al., 2019). In Egypt, despite the application of long-term control programs, some *S. mansoni* endemic foci still exist [King, 2010]. For effective disease control and routine diagnosis, there remains a critical need for improved technologies and more sensitive and cost-effective diagnostic tools (Lecky et al., 2014).

The Kato-Katz technique has long been the backbone of intestinal schistosomiasis diagnosis in endemic areas. As a quantitative method, it serves as a proxy for worm load in the human host and is widely used in disease mapping. However, its sensitivity is markedly decreased at low infection intensities where accurate detection is essential for transmission assessment (Lecky et al., 2014). In addition, stool sample collection is often challenging and uncomfortable for participants and the management of human fecal material is a logistical challenge in large-scale field studies (Zacharia et al., 2024).

Collecting of urine samples has the potential to be more applicable than other samples in detecting *S. mansoni* because it can be managed and obtained easily from individuals of all ages. Additionally, small amounts of cell-free circulating *S. mansoni* DNA can be found in excreted urine and reflects the existence of live worms or eggs (Hessler et al., 2017; Miller et al., 2024).

The molecular techniques have been reported for the detection of schistosomiasis DNA in human feces, sera, plasma and urine (Allam et al., 2009, Wichmann et al., 2009). Real-time PCR, enables the simultaneous amplification and detection of DNA in real-time with high specificity and sensitivity (Deepak et al.2007, Fuss et al., 2020). However, PCR-based assays are not well-suited for the current need for cost-effective and easily handheld devices (Abdel-Hafeez et al., 2015; Meurs et al., 2015).

In 2000, loop-mediated isothermal amplification (LAMP) emerged as a promising point of care (POC) technique. It amplifies DNA under isothermal conditions, using an enzyme with strand displacement activity (Notomi et al.,2000; Abdul-Ghani et al., 2012; Njiru, 2011). As a molecular technique, LAMP offers potential advantages, such as simplicity, speed and cost-effectiveness. It can be visually detected without specialized equipment based on the created turbidity due to the gene proliferation and the production of magnesium pyrophosphate ($Mg_2P_2O_7$); while in recent years, the use of SYBR Green dye facilitated this step, making it suitable for use in resource-limited settings (Rivero et al., 2017, Mwangi et al., 2018; Fernández et al., 2019).

The DNA extraction methods are the key for diagnosis of schistosomiasis by molecular techniques. Many commercial kits are available for schistosomiasis DNA extraction from various biological samples; however, their high cost may limit widespread use. Phenol–chloroform was one of the first widely used methods for extracting DNA, successfully allowing the recovery of fragmented ancient DNA (aDNA) from museum collections and Late Pleistocene animals with minimal loss (Bergallo et al., 2006; Barnett et al., 2012). It is particularly valued for its effectiveness in removing proteins and other contaminants an essential feature when working with degraded, low-yield aDNA samples. Consequently, this study aimed to assess the diagnostic performance of real-time PCR and LAMP assay for *S. mansoni* detection in urine samples, using a commercial kit and phenol chloroform DNA extraction methods.

2. Materials and methods

Study subjects

This study was conducted on schoolchildren (6–12 years) in Elsalahba School, Kafr El-Sheikh governorate, 120 km away from Alexandria. This governorate is still a low endemic area of *S. mansoni* while *S. haematobium* has been eradicated (Allam et al., 2018).

Ethical consideration

Ethical approval for the study protocol was granted by the Ethical Committee of the Medical Research Institute with the approval code of IORG0008812. Informed consents were secured from school guardians and children's parents. Positive cases for *S. mansoni* or other parasites were referred for conventional treatment.

Sample collection

In total, 200 schoolchildren were asked to submit stool samples. The collected samples were transported to the laboratory for microscopic examination. Urine samples were also obtained from a subset of children, who consented to participate in the study, and were stored at -20°C for further processing.

Kato-Katz

All stool samples were examined microscopically using the Kato-Katz technique (41.7 mg/slide, three slides/each sample) and eggs were counted according to Katz et. al. (1972).

Urine based real-time PCR and loop mediated isothermal amplification (LAMP)

Twenty five morning complete urine samples (not just the terminal urine) were collected from positive and negative *S. mansoni* cases with different infection intensities based on Kato-Katz results.

In addition, 10 urine samples were collected from age-matched healthy volunteers attending an urban primary health care unit in Alexandria, a non-endemic area for Schistosoma. All samples were processed using the real-time PCR and LAMP techniques. Urine DNA was extracted by two different methods; the urine DNA commercial extraction kit (Chongqing Biospes Co., Ltd., China) and a homemade phenol/chloroform method (Bergallo et al.,2006).

DNA commercial extraction kit

Briefly, each urine sample (10 ml) was centrifuged for 15 minutes at 14,000 rpm at a temperature of 4°C. The supernatant was carefully discarded, and the cell pellet was re-suspended in 200µL of buffer UB; 20 µl of Proteinase K solution (20 mg/ml) were added, mixed thoroughly, then 200 µL of Binding Solution CB were added and vortexed for thoroughly mixing, 100 µl of isopropanol were added to the mixture, vortexed, 500 µL of inhibitor removal solution IR were added to the mixture, then transferred to an adsorption column AC, centrifuged at 12,000 rpm for 30 seconds, and the waste solution was discarded, 500 µl of Rinse solution WB (ensuring that anhydrous ethanol had been added before use) was added, then centrifuged at 12,000 rpm for 30 seconds and the waste discarded. The wash was repeated and discarded as before. The column was returned to an empty collection tube and centrifuged at 13,000 rpm for two minutes, then transferred to a clean centrifuge tube. 30 µl of Eluent Buffer EB (pre-warmed at 65-70°C) were added to the center of the adsorbent membrane. It was left to stand at room temperature for 3-5 minutes, and then centrifuged at 12,000 rpm for one minute. Each extracted DNA was stored at -20°C until use.

Phenol-chloroform DNA extraction

Each urine sample (10 ml) was centrifuged for 15 minutes at 12,000 rpm. The supernatant was discarded, and the cell pellet was suspended in 700µl of triazol and 25µl of 3 M sodium acetate pH 5. Vortexed for 1 minute, left for 5 minutes, 300µl of chloroform were added and vortexed again for 1

minute. Incubated in ice for 15 min and centrifuged for 10 min at 12,000 rpm at room temperature. The aqueous layer was transferred to 1.5 ml screw-cap plastic tube containing 250 µl of isopropanol and incubated at −20 °C for 1 hour. Each sample was centrifuged for 15 min, 12000 rpm at 4 °C in order to precipitate DNA. The supernatant was removed, and the pellet was suspended in 500 µl of 70 % ethanol, vortexed and centrifuged for 2 min at 13,000 rpm at room temperature. The supernatant was removed again and the previous step was repeated, the remaining ethanol eliminated by a quick centrifugation. The pellet was air-dried in a half-open tube for at least 30 min, suspended in 20 µl of nuclease free water and stored at −20 °C until use.

Real-time PCR

S. mansoni detection and data analysis were conducted using Agilent Stratagene Mx3000P™ Real-time PCR system. The reaction mixture was prepared for both urine DNA extraction methods. Briefly, each reaction included a set of *S. mansoni* forward and reverse primers: SmF GAGATCAAGTGTGACAGTTTTGC and SmR CAGTGCGCGCGTCGTAAGC, GenBank accession AY157173, targeting the 28 S rDNA region from *S. mansoni* were selected to amplify a fragment of 350 bp [25]. The reaction mixture included 4 µl of extracted DNA from each urine sample, 1 µl of each *S. mansoni* primer (forward and reverse), 12.5 µl SYBR Green universal PCR master mix, water free nuclease was added in the reaction tube to a final volume of 25 µl. The real-time PCR was then performed under the thermal profile as follows: an initial denaturation step at 95°C for 2 min was firstly carried out, then 40 cycles of amplification were performed (95°C for 15 sec and 60°C for 60 sec). Melting curve analysis was done to determine the purity and specificity of the amplified product. It was performed after each PCR run, by increasing temperature slowly from 60°C to 95°C for one min, then at 55°C for 30sec and finally at 95°C for 30 sec.

LAMP procedures

The LAMP assay targeted conserved regions of *S. mansoni* DNA using a specific primer sets (Sm1-7), including external (F3, B3) and internal primers (FIP, BIP), (Table 1) (Abbasi et al., 2010, García et al., 2021). The reaction mixture (25 µl total volume) contained template DNA (4.0 µL), primers contained (2 µl of each FIP and BIP and 3 µl of F3 and B3 outer primers), 5 µl of LAMP reaction mix, TS LAMP Green dye (0.5 µl), BST III DNA polymerase (2.0 µl), and nuclease-free water (8.5 µl). Incubation was performed at 63°C for 60 minutes under isothermal conditions. Amplified DNA was visualized under UV light (320 nm) after 20-minute electrophoresis on a 2% agarose gel and photographed. Direct detection was performed by naked-eye observation before and after adding a 1:10 dilution of SYBR Green I dye (Invitrogen, Carlsbad, CA), with a color change from orange to yellowish green indicating a positive LAMP reaction.

Positive and negative controls were used in each run of real-time PCR and LAMP techniques. Positive control was selected from positive cases of heavy infection intensity as diagnosed by Kato-Katz (1 ng/µl *S. mansoni* DNA as measured by NanoDrop ND-1000 spectrophotometer). M, molecular weight marker was used in each run of LAMP assay.

Table 1. Primer sets targeting selected sequences for *S. mansoni* cell-free DNA amplification by LAMP

Primer set	***Primer position (length bp)**	**Primer sequence 5′→3′**	**GenBank Accession. No.**
S. mansoni (Sm1-7)	F3	GAT CTG AAT CCG ACC AAC CG	L27240)
	B3	AAC GCC CAC GCT CTC GCA	
	FIP: F1c + F2	AAATCCGTCCAGTGGTTT TTTT GAAAATCGTTGTATCTCCG	
	BIP: B1c + B2	CCGAAACCACTGGACGGA TTTT TATTTTTAATCTAAAACAAACATC	

* F3 = forward external primer; B3 = backward external primer; FIP = forward internal primer composed of the F1c and F2 primers sequence; BIP = backward internal primer composed of the B1c and B2 primers sequence.

Statistical analysis

Data were analyzed using IBM SPSS for Windows, Version 20.0 (Armonk, NY: IBM Corp). The statistical program was utilized for both data presentation and statistical analysis of the results. For descriptive analysis, the prevalence of *S. mansoni* detected by different methods was expressed as percentages; egg counts by Kato-Katz were used to express infection intensity and geometric mean egg count (GMEC) [28]. Confidence interval (CI) at 95% using Med Calc 12.4.0 (Med Calc Software, Belgium) was applied as measures of central tendency and dispersion respectively for normally distributed quantitative data. Association between categorical variables was tested by Fisher exact tests, they were considered significant when $p<0.05$. The real-time PCR and LAMP assay sensitivity, specificity, PPV, and NPV was evaluated by establishing a reference standard incorporating all the positive cases by Kato-Katz, real-time and LAMP urine DNA commercial extraction kit and LAMP urine phenol/chloroform extraction methods (Kotz et al., 2006),

Results

S. mansoni infection rate and intensity by Kato-Katz

Using Kato-Katz, among the 200 schoolchildren, *S. mansoni* infection was detected in 36 cases (18%). Infection intensity revealed 38.9%, 55.55% and 5.55% of cases had light, moderate and heavy infection respectively, with an overall GMEC of 112 epg (Table 2).

Table 2. *S. mansoni* intensity among the 36 positive schoolchildren **by** Kato-Katz

Intensity of infection (epg)*	**Infected children**		**GMEC (95% CI)**
	No.	%	
Light (<100 epg)	14	38.9	57 (43 - 74)
Moderate (100-400 epg)	20	55.55	157 (140- 176)
Heavy (>400 epg)	2	5.55	476 (279 - 811)
Total	36	100	112 (89 - 142)

*Infection intensity was categorized by eggs per gram (epg). GMEC: Geometric Mean Egg Count; CI: Confidence Interval.

Urine-based real-time PCR and LAMP techniques for the *S. mansoni* detection based on Kato-Katz results

Table 3 compares between real-time PCR and LAMP assays using the two extraction methods for detecting *S. mansoni* DNA in urine samples among different infection intensities determined by the Kato-Katz method. The real-time PCR and LAMP commercial kit detected *S. mansoni* DNA in 100% of light, moderate, and heavy infections, indicating consistently high performance regardless of parasite load. In contrast, phenol/chloroform real-time PCR and LAMP demonstrated lower efficiency in light infection (75%, 62.5% respectively) and detected all cases of moderate and heavy infections (100%). The association between the detection rate and infection intensity showed that this method is more affected by parasite load and less sensitive at low intensities compared with the commercial kit.

Focusing on the Kato-Katz negative cases, real-time PCR commercial kit detected 80% of Kato-Katz negative samples corresponding to 40% by LAMP assay. However, real-time PCR and LAMP with phenol/chloroform extraction showed lower efficiency in Kato-Katz negative cases (0%, 20% respectively). Considering all cases (positives and negatives), there was no significant difference only

between real-time PCR commercial and Kato-Katz negative cases. All control volunteers gave negative results with all employed techniques.

Table 3. Real-time PCR and LAMP assays with commercial kit and phenol/chloroform for *S. mansoni* detection in urine samples compared to Kato-Katz infection intensity

Techniques	Positive Kato-Katz (n=20)								FET	P
	Light intensity (n = 8)		Moderate intensity (n = 11)		Heavy intensity (n = 1)		Negative Kato-Katz (n=5)			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)		
Real-time PCR Commercial kit										
Positive	8	(100)	11	(100)	1	(100)	4	(80)	4.167	0.244
Negative	0	(0)	0	(0)	0	(0)	1	(20)		
LAMP Commercial kit										
Positive	8	(100)	11	(100)	1	(100)	2	(40)	9.239*	0.009*
Negative	0	(0)	0	(0)	0	(0)	3	(60)		
Real-time PCR Phenol/Chloroform										
Positive	6	(75)	11	(100)	1	(100)	0	(0)	6.96	0.001*
Negative	2	(25)	0	(0)	0	(0)	5	(100)		
LAMP Phenol/Chloroform method										
Positive	5	(62.5)	11	100	1	(100)	1	(20)	11.319*	0.003*
Negative	3	(37.5)	0	(0)	0	(0)	4	(80)		

FET: Fisher Exact test. *p: p* value for comparing between different categories. *: Statistically significant at $p \leq 0.05$

Figure 1 shows that real-time PCR positive samples display characteristic amplification curves and distinct melting peaks, while negative samples show no amplification signals or melting peaks throughout the reaction. LAMP positive and negative cases were determined by the naked eye (Fig.2).

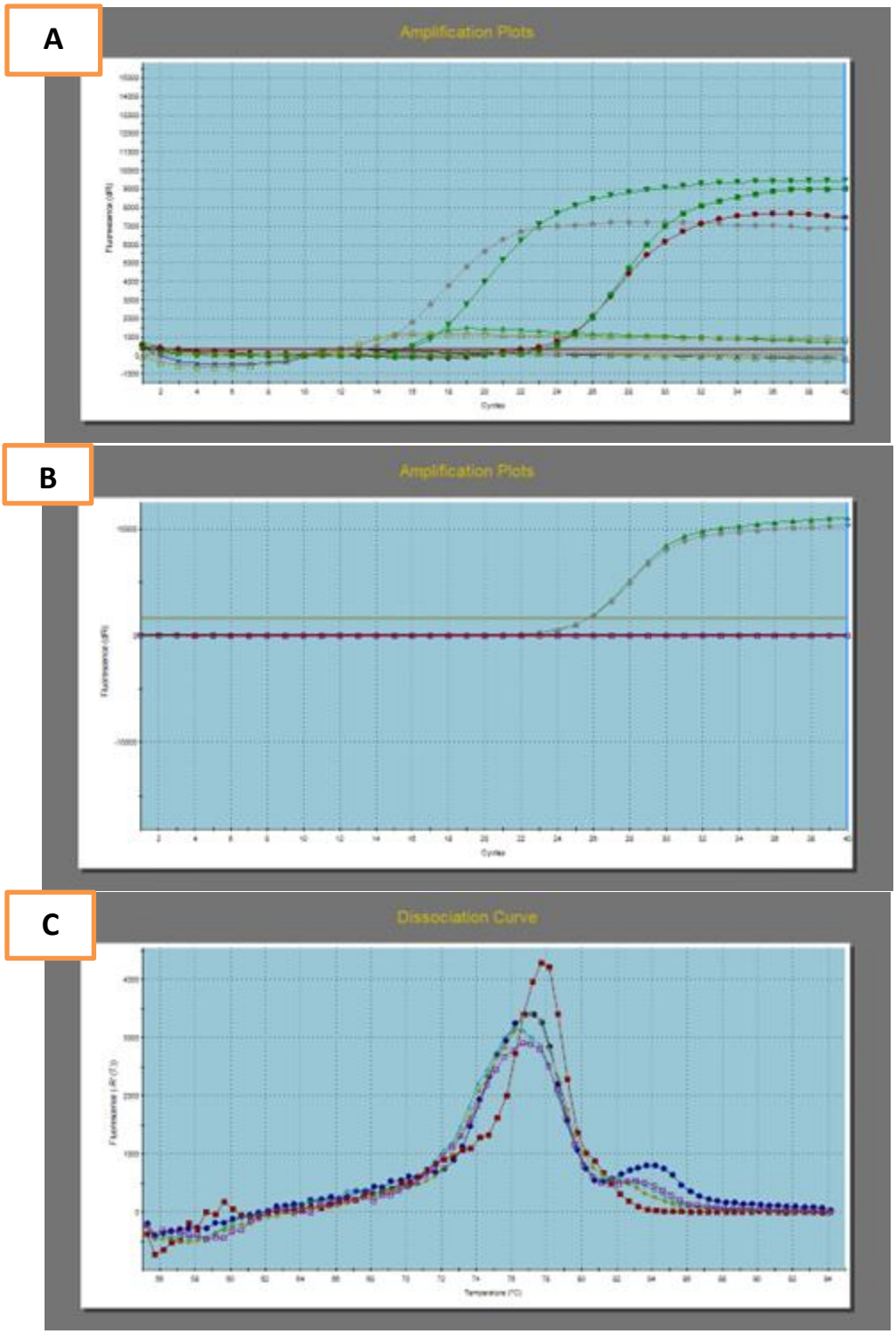


Figure 1 Amplification plot of real-time PCR representative samples using a primer pair specific to *S. mansoni*. A. Amplification plot of urine real-time PCR commercial kit. B. Amplification plot of urine real-time PCR phenol/chloroform. Positive samples display characteristic amplification peaks (Ct range: 16 to 26) and distinct melting curve with melting temperatures of 76-78ºC (C).

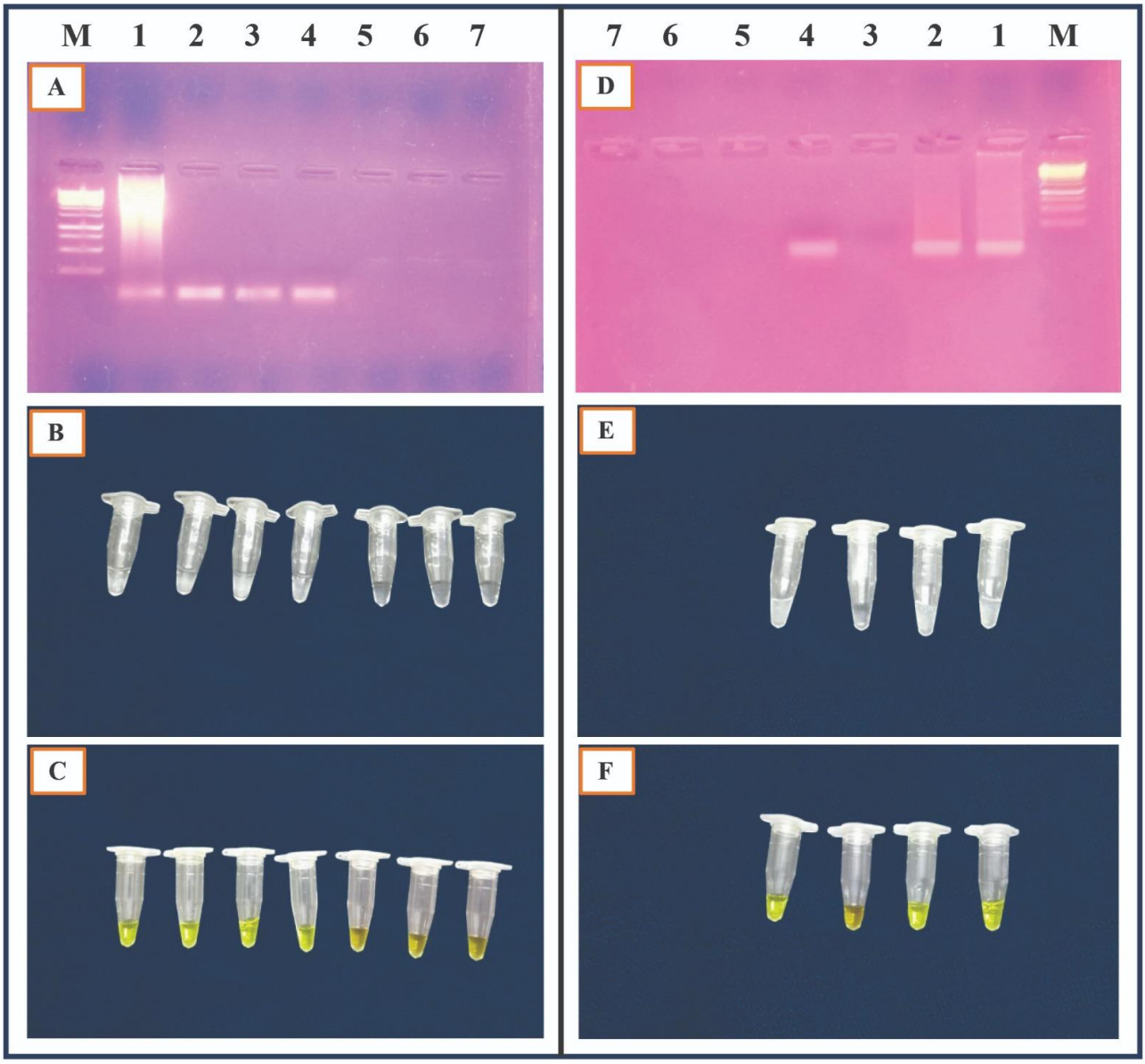


Figure 2 Representative samples of the urine LAMP DNA commercial extraction kit and the phenol/chloroform extraction method for *S. mansoni* detection. (A) Gel electrophoresis of LAMP products using urine DNA extracted with the commercial kit; lane M: molecular marker; lanes 1–4 are positive for *S. mansoni* corresponding to tubes 1-4, while lanes 5–7 are negative corresponding to tubes 5-7 (B & C). (D) Representative samples of gel electrophoresis of LAMP products using DNA extracted with the phenol/chloroform method; M: molecular marker; lanes 1, 2, and 4 are positive for *S. mansoni*, corresponding to 1, 2 and 4 tubes. Lane 3 is negative, corresponding to tube 3 (E & F). Turbid tubes are *S. mansoni* positive (B &E). After adding SYBR Green, a yellowish-green color indicates a positive result, whereas an orange color indicates a negative result (C&F).

Diagnostic performance of the employed methods

Based on the calculated gold standard, which combined results from Kato–Katz, real-time PCR, and LAMP (24 true positives; 20 detected by Kato–Katz, plus 4 additional cases detected by real-time PCR, including two identified by the LAMP commercial kit), and a prevalence rate of 96% (24/25 x100), Kato–Katz demonstrated a sensitivity of 90%, a negative predictive value (NPV) of 60%, and an overall accuracy of 92%. In comparison, the real-time PCR urine commercial kit demonstrated the highest sensitivity (100%), NPV (100%), and accuracy (100%), followed by LAMP DNA commercial kit of 91.6% sensitivity, 33.3% NPV and 92% accuracy. The real-time PCR and LAMP DNA phenol/chloroform method had the lowest performance, with 75% sensitivity, 14% NPV and 76% accuracy (Table 4).

The **Table 4.** Performance parameters of the real-time PCR and LAMP using the two DNA extraction me methods for *S. mansoni* detection in the 25 examined urine samples*

Techniques	Positives No. (%)	Sensitivity 95% CI	Specificity 95% CI	NPV 95% CI	PPV 95% CI	Accuracy 95% CI
Kato-Katz	20 (80)	83.33% (62.62-95.26)	100% (2.50 -100)	20% (9.27-37.95)	100% (83-100)	84% (63.92-95.46)
Real-time PCR (DNA Commercial kit)	24 (96)	100%	100%	100%	100%	100%
		(85.75-100.)	(2.50-100)	(2.50-100)	(85.75-100)	(86.28-100)
LAMP DNA (Commercial kit)	22 (88)	91.67 (73.0 - 98.97)	100% (2.5-100)	33.3% (11.71-65.33)	100% (84.56-100)	92.0% (73.97-99.02%)
Real-time PCR DNA (Phenol/ Chloroform)	18 (72)	75% (53.29-90.23)	100% (2.50-100)	14.29% (7.69-25)	100% (81.47-100)	76.00% (54.87-90.64)
LAMP DNA (Phenol/ Chloroform)	18 (72)	75% (53.29-90.23)	100% (2.50-100)	14.29% (7.69-25)	100% (81.47-100)	76.00% (54.87-90.64)

NPV: Negative predictive value, PPV: positive predictive value. * Healthy controls were not included in the table because all samples tested negative, demonstrating the specificity of the diagnostic methods.

Discussion

Despite ongoing control efforts, schistosomiasis continues to contribute to substantial morbidity and mortality in endemic areas, calling for accurate diagnostic methods to support precise detection and eventual eradication by 2030 (WHO, 2025). In this context, to overcome some diagnostic limitations of the Kato-Katz technique, particularly, in low-intensity infections and the challenges associated with fecal sample collection, this study compared two DNA extraction methods from urine samples using real-time PCR and LAMP assay to develop simple, cost-effective, and sensitive methods for *S. mansoni* diagnosis in low-endemic areas.

The results of the current study using Kato-Katz revealed that *S. mansoni* was detected in 36 cases with a corresponding prevalence of 18%. The intensity of *S. mansoni* infection was categorized as 38.9% light, 55.6% moderate, and 5.6% heavy. Despite the relatively low prevalence, moderate-intensity infections were predominant, with an overall GMEC of 112 epg. The low prevalence in this study compared to previous work in the same area reflects the effectiveness of treatment campaigns targeting school-aged children (Allam et al., 2022). However, the relatively high GMEC indicates that transmission persists within the population and underscores the risk of resurgence if treatment programs and continuous monitoring are halted.

In this study, Kato-Katz negative and positive cases with different infection intensities were used to assess the effectiveness of the urine-based real-time PCR and LAMP assays for *S. mansoni* detection. Among 25 urine samples (20 Kato-Katz positive and 5 negative), real-time PCR and LAMP with commercial extraction kit identified all Kato-Katz positive cases with different infection intensity, indicating consistently high performance regardless of parasite load. In contrast, phenol/chloroform extraction missed some light infections, showing that this method is less sensitive compared with the

commercial kit. Conversely, Worrell et al. (2015) reported superior diagnostic performance of this method over the commercial kit.

Regarding the Kato-Katz negative cases, both real-time PCR and LAMP with the two extraction methods, detected *S. mansoni* positive cases in urine samples that were negative by Kato-Katz, underscoring their ability to identify infections overlooked by microscopy, consistent with reports in many studies [23, 33]. Notably, real-time PCR detected the highest number of positives within the Kato-Katz negative cases compared to LAMP (80% vs 40 %), signifying its higher efficiency for identifying missed cases. However, Fernández-Soto et al. (2019) reported a greater number of positive results by SmMIT-LAMP than using PCR for S. mansoni DNA detection in urine samples (Fernández et al., 2019).

Confirming the current results, the diagnostic performance revealed the higher sensitivity of real-time PCR compared to LAMP assay regardless of the extraction method. The commercial DNA extraction kit used with the two assays demonstrated highest sensitivity, negative predictive value (NPV), and accuracy compared to the phenol/chloroform method. The possible explanations of the difference in performance between the two extraction methods may be due to variations in DNA recovery efficiency, or urinary inhibitors. However, both urine-based assays proved effective, especially when egg counts were below the Kato-Katz detection limit (<8 epg; equivalent to one egg per three slides).

In this study, urine-based real-time PCR and LAMP assays highlight urine as a reliable sample for *S. mansoni* detection. This backs earlier findings that PCR and LAMP can sensitively detect *S. mansoni* cell-free DNA in urine, even at 0.1 fg levels. Furthermore, urine samples can be collected either in liquid form or as dried spots on filter paper, facilitating their ease of transportation and storage for field studies (Keikha et al., 2018, Enk et al., 2012; Sarhan et al., 2015).

Ultimately, despite the relatively high cost and complexity of the real-time PCR technique, its high performance makes it suitable for targeted field applications alongside Kato-Katz, particularly in negative individuals, post treatment situation, true prevalence estimation, and when disease mapping and elimination are cardinal goals. Considering the LAMP assay as a low-cost and single-tube molecular technique, it presents a valuable complementary tool to the Kato-Katz for *S. mansoni* diagnosis in low-resources settings. Its performance may be further improved through optimizing incubation time, adjusting DNA quantity, refining extraction steps, using other primers and modifying phenol/chloroform preparation (Mukhopadhyay et al., 1993, Al-Soud et al. 2001). This method costs approximately half the commercial kit, supporting the findings by Bergallo et al. (2006) that it can serve as a low-cost, effective option.

Conclusion

Urine samples are promising for detecting *S. mansoni* through trans-renal DNA. Urine- based real-time PCR revealed a high diagnostic performance for *S. mansoni*. The urine-LAMP using commercial extraction kit could be acknowledged as a good molecular technique offering a simple and rapid method. The phenol/chloroform DNA extraction exhibited promising and cost-effective diagnostic efficiency in light, moderate and heavy infection; nevertheless, further refinement is required to improve its reliability. Further large-scale molecular studies on urine samples and extraction methods are still needed.

Study limitations

The small sample size (25 children) might be a limitation of the study; however, it serves as a valuable starting point and foundation for future research on urine-based molecular assays.

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Conflicts of interest None.

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Declaration of competing interest

The authors declare that they have no competing interests and this manuscript has not been submitted to any other journal in Egypt or abroad. Alexandria University's code of ethics and rules of academic integrity had been followed.

Data availability statement

All relevant data are included in the manuscript.

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