Determination of Infection Prevalence of *Schistosoma mansoni* after MDA: Comparison of LAMP and PCR Diagnosis

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ABSTRACT

Schistosoma mansoni is one of the main contributors of schistosomiasis accounting for more than 90 million people in 54 countries, where children are more susceptible group. The eradication of low-level persistent infections after mass drug administration (MDA) requires a diagnostic test that can be performed in the field and clinic with ease and greater sensitivity. To address this, we evaluated 111 urine samples collected from Zambian school children (7 - 15 years) by point-of-care (POC) diagnosis through loop-mediated isothermal amplification (LAMP) and compared its sensitivity and specificity with polymerase chain reaction (PCR) for two extraction methods (LAMP-PURE and Qiagen). The sensitivity of LAMP amplification (100%) was greatest when paired with Qiagen extraction. Also, LAMP detected 10% (LAMP-PURE extraction) and 16% (Qiagen extraction) more positive S. mansoni infection compared to PCR. But, LAMP-PURE extraction resulted in false negatives (6%) and requires attention before it can be used as a field extraction kit. Overall infection prevalence was 77% with PCR and almost 93% with mansoni LAMP assay. We have demonstrated the efficacy of LAMP in detecting low level infection especially after MDA. LAMP can be added as a POC as part of integrated diagnosis for surveillance of schistosomiasis prevalence in endemic regions.

BACKGROUND

- London Declaration 2020 aims to implement interventions necessary to control or eliminate several Neglected Tropical Diseases (NTD), including **Schistosomiasis**.(1)
- Over 200 million individuals are currently infected by the disease and more than 700 million are at risk of getting infected.(2)
- Due to mass drug administration (MDA) control intervention, the detection limit for remaining or new infection posses challenges for gold-standard (WHO recommended) tests and are often missed. (3)
- Polymerase chain reaction (PCR) has been demonstrated to detect *Schistosoma mansoni* specific repeat DNA fragments captured on filter paper through urine filtration. This approach is devoid of stool testing and reliance on parasitic eggs.(4)
- The inherent technological limitation of PCR makes it unusable in the field. Whereas loop-mediated isothermal amplification (LAMP) can be used as a point-of-care (POC) assay for field diagnostics. (5)
- LAMP utilizes four primers which amplify 6 different regions of the target DNA, and this occurs at one constant temperature. Positive reactions will yield changes in color or turbidity. (6)

OBJECTIVE

We have evaluated the positive infection for *S. mansoni* from filtered urine samples collected from Zambian school children after MDA through LAMP and compared its sensitivity and specificity with PCR.

MATERIALS and METHODS

- The study was conducted in the Chongwe and Siavonga districts of Zambia.(7)
- Participants consisted of 111 school children between the ages of 7 and 15.(7)
- Urine samples were collected from all participants after one round of MDA.
- Dried urine filter papers were used as the source of parasite DNA.
- DNA extraction was performed using the gold standard Qiagen kit and the rapid extraction LAMP-PURE kit.

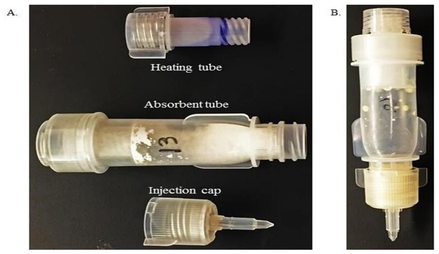

Fig. 1: LAMP-PURE DNA extraction kit: (A) Three components of the extraction kit. (B) All attach components after DNA extraction and representation of a closed environment rapid DNA extraction.

❖ Workflow for PCR and LAMP:

Fig. 2: PCR Workflow

Fig. 3:LAMP Workflow

❖ PCR for detection of *S. mansoni:*

- Samples were combined with master mix, water, magnesium, sequence specific primers, deoxynucleotide triphosphates (dNTPs), and *taq* polymerase.
- PCR amplification occurred in a thermal cycler, with subsequent visualization of amplification by gel electrophoresis.

❖ LAMP amplification for *S. mansoni*:

- Samples were mixed with master mix, sequence specific primers, dNTPs and *Bst* polymerase.
- LAMP amplification occurred at one temperature, with subsequent visualization by fluorescent dye and by gel electrophoresis.

❖ Advantage of LAMP

- LAMP is an isothermal assay, meaning that the reaction takes place at one constant temperature. This removes the need for a thermal cycler.
- LAMP is more resistant to inhibitors than PCR. This is due to the *Bst* polymerase.
- Sensitivity of LAMP has been demonstrated to be greater than that of PCR.(8)
- LAMP reactions occur quicker than PCR and remove the need for special reagents. This saves time and money.

RESULTS

Table 1. Detection of positive and negative infection by PCR and LAMP for *Schistosoma mansoni* for both Qiagen and LAMP-PURE extracted DNA

N: 111 samples	PCR positive	PCR negative	LAMP positive	LAMP negative
Qiagen	**86**	25	**104**	7
LAMP-PURE	86	25	97	14

- When using PCR amplification, both extraction methods yielded the same number of positives and negatives.
- LAMP detected 18 (Qiagen) and 11 (LAMP-PURE) more positive infections compared to PCR for *S. mansoni*.

Table 2. Diagnostic comparison of amplification efficacy by PCR and LAMP for Qiagen and LAMP-PURE extracted DNA for *Schistosoma mansoni*

LAMP-Qiagen	PCR-Qiagen	
	Positive	Negative
Positive	86	18
Negative	0	7

PCR LAMP-PURE	PCR-Qiagen	
	Positive	Negative
Positive	65	21
Negative	21	4

LAMP_ LAMP_PURE	PCR-Qiagen	
	Positive	Negative
Positive	80	17
Negative	6	8

LAMP_ LAMP_PURE	LAMP-Qiagen	
	Positive	Negative
Positive	91	6
Negative	13	1

- LAMP on Qiagen extracted samples yielded 18 more positive reactions which were negative by PCR for Qiagen extracted samples.
- LAMP-PURE extraction technique did not produce similar results to Qiagen extracted samples for both PCR and LAMP.

Table 3. Agreement statistics estimation (Kappa coefficient and Bowker symmetry test) comparing species-specific DNA amplification by PCR and LAMP for both Qiagen and LAMP-PURE extracted DNA for *S. mansoni*

Comparison of diagnostic tests	Kappa coefficient		Bowker's symmetry test©	
	Degree of agreement	95% CI	Symmetry of disagreement	P value$^{\Psi}$
PCR-Qiagen vs. LAMP-Qiagen	0.38	0.17 – 0.58	18	**0.0001***
PCR-Qiagen vs. PCR_LAMP-PURE	-0.08	-0.25 – 0.09	0	1.0000
PCR-Qiagen vs. LAMP_LAMP-PURE	0.30	0.08 – 0.51	5.26	**0.0218***
LAMP-Qiagen vs. LAMP_LAMP-PURE	0.01	-0.17 – 0.19	2.58	0.1083

©Bowker's Symmetry test = this test checks for symmetry in 2-way tables and the test decision is based on a X^2 approximation of the distribution of the test statistic
$^{\Psi}$ = α level was set at 0.05
•= Significant

- Test positives for PCR-Qiagen were highly likely to be the same with LAMP-Qiagen, but more sensitivity with LAMP-Qiagen.
- PCR-Qiagen and PCR_LAMP-PURE were highly unlikely to yield the same result.
- LAMP-Qiagen positives may be unlikely to be the same as LAMP_LAMP-PURE results.

CONCLUSION

- The infection prevalence was 77% (86/111) with PCR and almost 93% (104/111) with LAMP for *S. mansoni*.
- LAMP detected 10% (LAMP-PURE) to 16% (Qiagen) more positives *S. mansoni* infection compared to PCR.
- Our study is consistent with previous studies about LAMP being more sensitive than PCR.(8)
- LAMP-PURE extracted DNA resulted in some discrepancies for both PCR and LAMP amplification.
- We found that LAMP is more sensitive in detecting low level infections, especially after MDA. This is highly important in determining the acceptability of LAMP as a field diagnostic technique.
- LAMP can be implemented as an integrated diagnostic approach (POC test) for surveillance and to determine infection prevalence in endemic regions to evaluate the efficacy of control programs.
- Utilizing LAMP will help achieve one of the goals of the London Declaration of 2020. This will help stakeholders make informed decision for control intervention.
- **Molecular xenomonitoring** via isothermal assays can be used to determine the prevalence of infected snails. They can be easily used to detect parasitic larvae in the intermediate host.

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