Cost effectiveness of LAMP Test for Molecular Diagnosis of Human Schistosomes

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ABSTRACT

Schistosomiasis in Africa is an ongoing public health problem which in recent times has attracted a major campaign to control the disease. It is caused by two major species, *Schistosoma mansoni* and *S. haematobium*, which often cause concurrent infections in the human population. Due to control efforts, the issue of diagnostic sensitivity has become more critical in the assessment of program effectiveness and the World Health Organization has drawn attention to the need for field-applicable tests with high specificity and sensitivity. To address that, we have evaluated the amplification of *S. mansoni* and *S. haematobium* by loop-mediated isothermal amplification (LAMP) from field-collected filtered urine samples collected from school children in Zambia. We have used four DNA extraction techniques (Qiagen, LAMP-PURE (LP), Chelex, and heating) to determine their impact on LAMP sensitivity and specificity along with cost analysis and person-time involvement for each approach. Qiagen and LP extraction both detected all positive infections, but Qiagen extraction is more cost-effective than LP. DNA extraction by LP is the fastest (average 20 min.) compared to the other three methods, although it is the most expensive including amplification ($9.35 compared to $4.90 for heating extraction and amplification). Chelex extraction is slower and simpler than LP and detected 20% more positive infection than heating. Heating extraction is very fast, inexpensive, and simple to perform. However, LAMP amplification for heating-extracted samples resulted in false-negatives, possibly indicating the presence of inhibitor(s). We have demonstrated the sensitivity, cost-effectiveness and time requirement of LAMP for detection of dual schistosome parasites from field collected urine samples. LAMP can be used as a point-of-care (POC) test for surveillance and assessing success of control intervention in Zambia as part of their ongoing local schistosomiasis control program.

BACKGROUND

- Schistosomiasis is caused by blood parasites called schistosomes.
- At least 230 million people are currently infected[1], mostly in sub-Saharan Africa.
- The most common species are *Schistosoma mansoni* and *S. haematobium*.[1]
- Children bear the highest infection prevalence and suffer from delayed physical and cognitive development as a result of infection.[2]
- A field-usable diagnostic test is needed to monitor disease prevalence, especially after Mass Drug Administration (MDA) in a resource-limited environment.

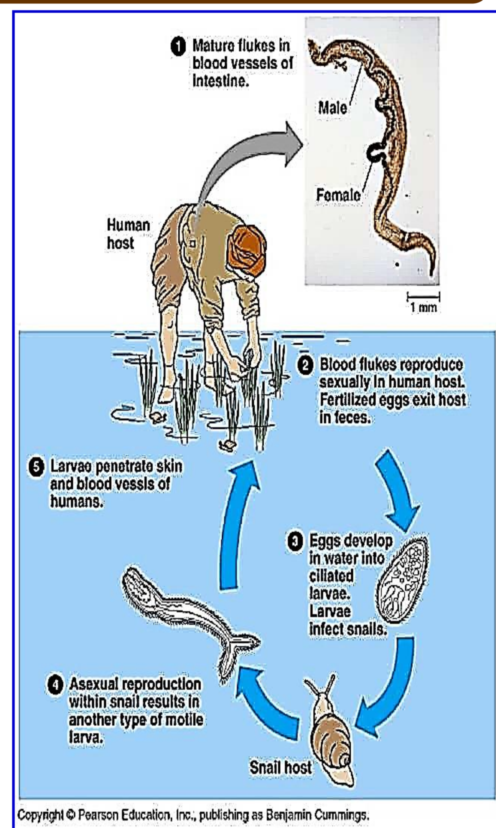

Figure 1: *S. mansoni* life cycle.

- Loop-mediated isothermal amplification (LAMP) has been used for the diagnosis of malaria, tuberculosis, and other infectious diseases and is a highly sensitive, specific, and rapid isothermal test.
- Using a strand displacement mechanism, LAMP can amplify DNA fragments at a constant temperature independent of expensive equipment.
- There is a lack of data regarding LAMP's cost-effectiveness, time requirement from extraction to detection, and amplification efficiency for different DNA extraction methods.

OBJECTIVE

- **Detect *S. mansoni* and *S. haematobium* infection via LAMP amplification from DNA extracted by four extraction techniques from a single urine specimen.**
- **Statistically evaluate sensitivity, specificity, cost effectiveness, and time requirement for LAMP amplification for four different DNA extraction methods.**

MATERIALS and METHODS

- Urine samples were collected from school children aged 9-13 years from the Chongwe and Siavonga districts of Zambia after one round of MDA.[5]
- Urine samples were filtered through filter paper, dried, sealed in individual plastic bags with a desiccant, and shipped to U.S.A.

Table 1: Information of samples used in this study. The identification of samples was determined by PCR amplification.

Combination	# of samples
S. mansoni + / *S. haematobium* +	8
S. mansoni - / *S. haematobium* -	7
S. mansoni + / *S. haematobium* -	8
S. mansoni - / *S. haematobium* +	7
Total	30

Figure 2: Four different DNA extraction methods.

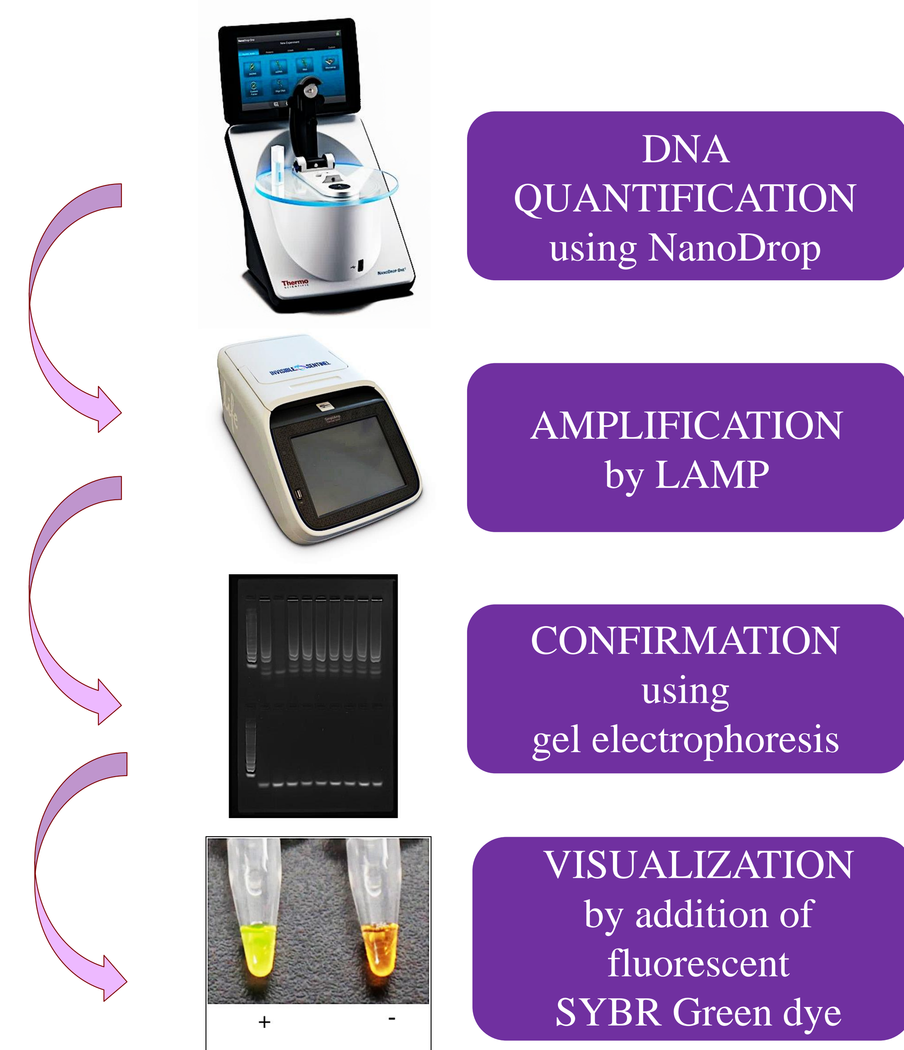

Figure 3: LAMP amplification workflow.

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Forward Primer
5'gatctgaatc cgaccaaccg gatctgaatc
3'ctagacttag gctggttggc ctagacttag

  cgaccaaccg ttctatgaaa atcgttgtat
  gctggttggc aagatacttt tagcaacata

  ctccgaaacc actggacgga gagagcgtgg
  gaggctttgg tgacctgcct ctctcgcacc

  gcgttaatat 3'
  cgcaattata 5'
Reverse Primer
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```
S. haematobium DRa 1-F
5'gatctcacct atcagacgaa acaaagaaaa
3'ctagagtgga tagtctgctt tgtttctttt

  ttttaaaatt gttggtggaa gtgcctgttt
  aaaattttaa caaccacctt cacggacaaa

  cgcaatatct ccggaatggt tggtcgtatc
  gcgttataga ggccttacca accagcatag

  gttgtgaaaa ttgtttcata ttattggtga c 3'
  caacactttt aacaaagtat aataaccact g 5'
S. haematobium DRa 1-R
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Figure 4: *S. mansoni* (left) and *S. haematobium* (right) cell-free repeat DNAs.

RESULTS

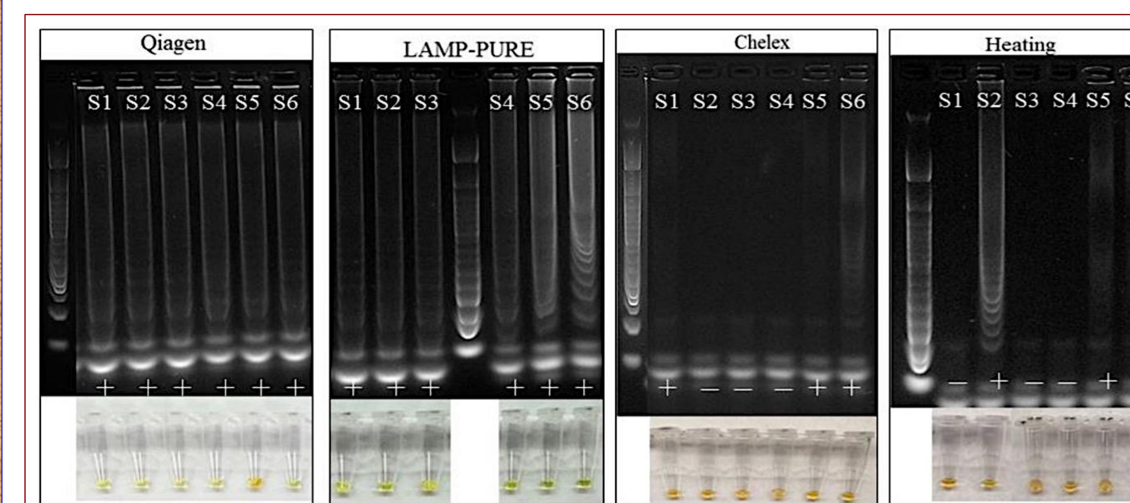

Figure 5: LAMP results after adding SYBR Green immediately after amplification compared against gel electrophoresis picture for amplification of cell-free repeat DNA for *Schistosoma mansoni*. Four different DNA extraction methods were compared for six samples and positive and negative amplification had been highlighted.

Table 2: Time requirement for DNA extraction for individual sample and cluster of samples by four different filter-based and non-filter-based methods.

DNA extraction type	Filter based/ non-filter based	Overall DNA yield (concentration)	Individual sample extraction time$	Cumulative time requirement (15 samples)	Amplification time
Qiagen QIAamp kit	Filter based	0.39ng/µl – 282ng/µl	43min.	1hr 25min. + 12hrs	2hr 32min.
LAMP-PURE*	Non-filter based	40ng/µl – 754ng/µl	21min.	2hr 56min.	2hr 32min.
Heating	Non-filter based	124ng/µl – 939ng/µl	30min.	2hr 9min.	2hr 32min.
Chelex	Non-filter based	106ng/µl – 559ng/µl	28min.	2hr 15min.	2hr 32min.

$ = Includes preparation, extraction, wait and quantification time.
* = Loop-mediated isothermal amplification – purified ultra-rapid extraction.

- LAMP-PURE requires the shortest time for extraction of a single sample.
- Extraction by heating is fastest for a cluster of samples.
- Qiagen extraction takes the longest due to the overnight wait period.

Table 3: LAMP amplification frequency for four different DNA extractions for *Schistosoma mansoni* and *S. haematobium*.

Schistosome species		LAMP			
		Qiagen QIAamp kit	LAMP-PURE	Chelex	Heating
S. mansoni	Positive	28 (93.3%)	28 (93.3%)	22 (73.3%)	21 (70%)
	Negative	2 (6.7%)	2 (6.7%)	8 (26.7%)	9 (30%)
S. haematobium	Positive	17 (56.7%)	21 (70%)	27 (90%)	27 (90%)
	Negative	13 (43.3%)	9 (30%)	3 (10%)	3 (10%)

- LAMP amplification for QIAamp and LP is consistent with PCR for both *S. mansoni* and *S. haematobium*.
- LAMP amplification is lower for Chelex and heating for *S. mansoni* and produced false-positives for *S. haematobium*.

Table 4: Cost analysis for four DNA extractions and LAMP amplification. Calculations are done based on single and multiple samples and also includes the cost of plastic supplies.

DNA extraction types	Extraction cost/ sample	LAMP test cost/ sample	Total for one sample	Total for 30 samples
Qiagen QIAamp kit	$4.00	$3.90	$7.90	$237.00
LAMP-PURE	$5.45	$3.90	$9.35	$280.50
Chelex	$2.60	$3.90	$6.50	$195.00
Heating	$1.00	$3.90	$4.90	$147.00

- Heating is the least expensive extraction method (extraction and amplification), followed by Chelex.
- LP extraction and amplification is the most expensive.

CONCLUSIONS

- LAMP amplification was achieved for both species of schistosome for DNA extracted by four different methods.
- Qiagen and LP extraction both detected 100% of positive infections, but Qiagen extraction is more cost effective than LP.
- DNA extraction by LP is the fastest compared to other three methods, but it is the most expensive.
- Chelex extraction is slower and simpler than LP and detected 20% more positive infection than heating.
- Extraction by heating is also very fast, inexpensive and arguably the simplest to perform. However, LAMP performed on heating-extracted samples resulted in many false-negative results, possibly indicating the presence of LAMP inhibitor(s).

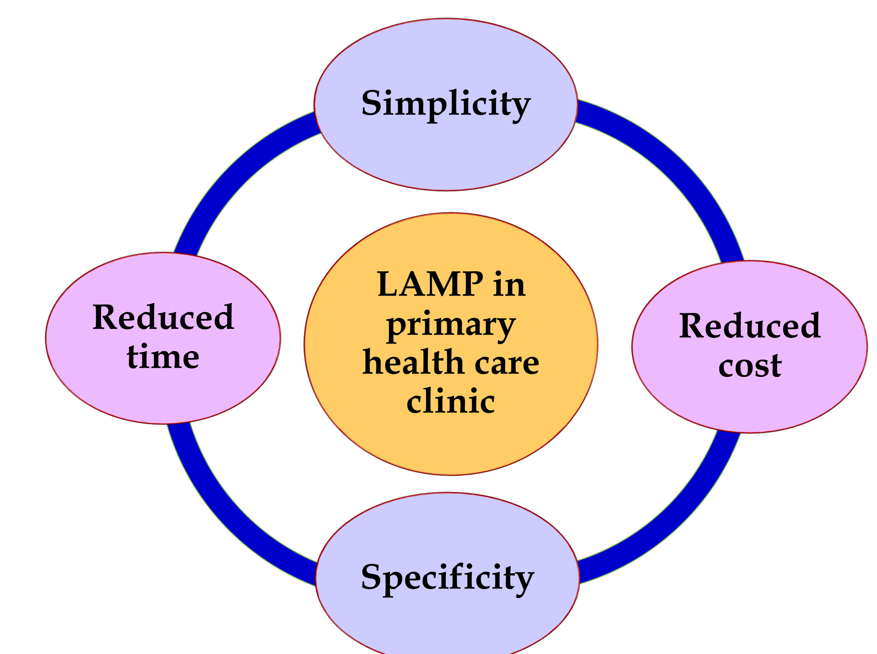

Figure 6: Advantages of LAMP in a Point-of-Care Setting.

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