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Field and laboratory comparative evaluation of a LAMP assay for the diagnosis of urogenital schistosomiasis in Cubal, Central Angola

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Abstract

OBJECTIVE To evaluate the performance of Rapid-Heat LAMPellet assay in field conditions for diagnosis of urogenital schistosomiasis in an endemic area in Cubal, Angola, and to assess the reproducibility in a reference laboratory.

METHODS A total of 172 urine samples from school-age children were tested for microhaematuria, microscopic detection of *Schistosoma haematobium* eggs and LAMP for DNA detection. Urine samples were stored in a basic equipped laboratory. Field-LAMP tests were performed with and without prior DNA extraction from urine samples, and the results were read by turbidity and by colour change. When field procedures were finished, samples were sent to a reference laboratory to be reanalysed by LAMP.

RESULTS A total of 83 of 172 (48.3%) were positive for microhaematuria, 87/172 (50.6%) were microscopy-positive for *S. haematobium* eggs detection, and 127/172 (73.8%) showed LAMP-positive results for detecting *S. haematobium* using purified DNA and 109/172 (63.4%) without prior DNA extraction. MacNemar's test showed a statistical significant relation between LAMP results and microscopy-detected *S. haematobium* infections and microhaematuria ($P < 0.001$ in both cases), respectively. When samples of purified DNA were reanalysed in a reference laboratory in Spain using the same LAMP methodology, the overall reproducibility achieved 72.1%.

CONCLUSIONS The ease of use, simplicity and feasibility demonstrated by LAMP assay in field conditions together with the acceptable level of reproducibility achieved in a reference laboratory support the use of LAMP assay as an effective test for molecular diagnosis of urogenital schistosomiasis in endemic remote areas.

keywords Loop-mediated isothermal amplification (LAMP), *Schistosoma haematobium*, diagnostic accuracy, microscopy, microhaematuria, Angola

Introduction

Urogenital schistosomiasis is a chronic, parasitic disease caused by the blood fluke *Schistosoma haematobium* affecting 112 million people with the highest prevalence in sub-Saharan Africa, Middle East and emerged in Mediterranean Europe [1–3]. The most common clinical presentations are haematuria, anaemia, dysuria and genital and urinary tract lesions, but in severe cases, it may also lead to infertility [4]. In chronic stages of the disease, the elimination of *S. haematobium* eggs eventually lead to squamous cell carcinoma of the bladder [5]. Moreover, urogenital schistosomiasis has also been shown to increase the risk of sexually transmitted diseases, including HIV infection, particularly in female genital schistosomiasis [6, 7]. In endemic countries, morbidity and mortality due to *S. haematobium* is a significant barrier to economic and social development [8].

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The microscopic identification of *S. haematobium* ova in filtered urine samples remains the 'gold standard' method for diagnosis of urogenital schistosomiasis, mostly under field conditions [9]. However, microscopy of filtered urine lacks sensitivity, especially in low-grade infections, leading to an underestimation of the true prevalence of the disease [10]. Moreover, egg count-based criteria cannot be carried out in the prepatence. The detection of microhaematuria has been widely used as a rapid diagnostic indicator for *S. haematobium* infection, especially in high transmission areas [11–13]. However, microhaematuria should not be considered as definitive diagnosis as it is a non-specific sign of urogenital schistosomiasis [4]. Antibody-based diagnostic tests are useful tools in several specific circumstances, but they suffer from many drawbacks [14]. On the other hand, circulating antigen-based detection methods have been developed to diagnose active schistosomiasis. The POC-CCA test showed good reliability in detecting some schistosome species, but poor accuracy for detecting *S. haematobium* [15]. The recently developed UCP-LF CAA urine assay has shown to be suitable for the diagnosis of *S. haematobium* and easy to perform but it needs some laboratory equipment for using under field conditions. Additionally, the major disadvantage of this test is that is not yet commercially available [16, 17].

Several molecular PCR-based methods have been used as alternatives to microscopy and serology for diagnosis urogenital schistosomiasis [18, 19]. These methods not only require expensive automated thermal cycler and associated PCR kits but also an acceptable level of training and infrastructure. A good alternative could be the loop-mediated isothermal amplification (LAMP) assay, a powerful nucleic acid amplification technique that combines simplicity, and elevated sensitivity and specificity [20]. At present, LAMP technology has all the characteristics required of a high-efficiency diagnostic assay along with simple operation for potential use in the clinical diagnosis of infectious diseases, including point-of-care testing under field conditions in developing countries [21,22]. Additionally, several LAMP assays have already been successfully used for detecting DNA from several schistosomes species, including *Schistosoma japonicum, Schistosoma mansoni* and *S. haematobium* [23–33].

The first LAMP assay for highly sensitive and specific detection of *S. haematobium* in human urine samples was recently reported by our group [34]. In that work, we demonstrated that the simple heating of urinary pellets for good-quality DNA extraction – the Rapid-Heat LAMPellet method – was effective for use in LAMP assays with regard to detect *S. haematobiun* in a number of clinical urine samples with proven infection with the parasite. The aim of this study was to evaluate the previously described LAMP assay under field conditions in a low-income urogenital schistosomiasis-endemic area. In addition, the reproducibility of the LAMP assay was evaluated in a reference laboratory.

Material and methods

Ethical considerations

The study was approved by the Ethical Review Board of Hospital Nossa Senhora da Paz (Cubal, Angola), the Vall d'Hebron University Hospital (Barcelona, Spain) and Ethics Committee of the Hospital Universitario de Salamanca, SACyL (Salamanca, Spain). Participation in the study was voluntary and with prior parental consent. All relevant authorities (village chiefs, school teachers and headmasters) were informed about the purpose and procedures of the study. Written informed consent was obtained from all parents and legal guardians. All students with confirmed infection received appropriate treatment with praziquantel. Procedures were followed the ethical standards laid down in the Declaration of Helsinki as revised in 2013.

Study area, population and samples collection

The study was conducted between February and July 2015 in the city council of Cubal, Benguela Province, Western-Central Angola, Africa. The city council of Cubal is formed of Cubal Sede and three communities namely Yambala, Capupa and Tumbulo with an overall estimated population of 322 000 with 151 000 (47%) children under 15 years old [35]. The study included a total of 252 school-age children ranging from 5 to 14 years old recruited in 10 schools of Cubal: two located in Cubal Sede, four in Yambala and four in Capupa. Pupils were randomly selected. Sex, age, school and community were recorded in a questionnaire.

Urine samples were requested from all children and collected at school between 10:00 and 14:00 h for optimum *S. haematobium* egg passage [36]. From each participant, a single individual sample of 15 mL was collected in a sterile container and then transported to the laboratory of the Hospital Nossa Senhora da Paz, Cubal Sede, for further processing. A total of 172 urine samples were finally included and tested in the study.

Microhaematuria and *Schistosoma haematobium* egg detection in urine samples

First, urine samples were screened for microhaematuria using reagent strips (COMBI-SCREEN®, Analyticon

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Biotechnologies, Germany). Then, a volume of 2 mL was reserved and stored at −20 °C for further DNA extraction for molecular analyses. The remaining volume of each urine sample was processed using the sedimentation by gravity method [37]. Next, all sediments obtained were examined for schistosome eggs under a microscope by qualified technicians and blinded to the microhaematuria results. No quantitative urine analysis was performed. The parasitological results were finally referred in terms of presence or absence of eggs in urine.

DNA extraction from urine

Aliquots of 2 mL of frozen urine samples were used for DNA extraction for molecular analysis. After thawing, each aliquot of 2 mL was divided into two new clean microcentrifuge tubes both containing 1 mL. Then, these aliquots were centrifuged at 2800 *g* for 5 min at room temperature to pellet the urinary sediment. Excess supernatant was discarded but maintaining a minimal volume of 100 μL to resuspend the urinary pellet at the bottom of the tube. For each one, a different DNA extraction method was performed as follows. For one of them, we used the i-genomic Urine DNA Extraction Mini Kit (Intron Biotechnology, UK) following the manufacturers' instructions and 2 μL of purified DNA thus obtained were added as template for LAMP amplifications. For the other, the resuspended urinary sediment was heated at 95 °C for 15 min and then briefly spin to pellet the debris. After this, 2 μL of the supernatant was used immediately as template for each LAMP reaction without any purification procedure.

LAMP assays for *Schistosma haematobium* diagnosis

Both purified DNA and heated urine from each of the two aliquots of all urine samples were analysed by 'conventional LAMP assay' and the 'Rapid-Heat LAMPellet method', respectively, following the same protocol described by Gandasegui *et al.* [34]. A set of six oligonucleotide primers (including loop primers) were used targeting eight regions in the sequence of *S. haematobium* ribosomal intergenic spacer (IGS) DNA retrieved from GenBank (Accession No. AJ223838). The reaction mixtures (25 μL) contained 1.6 μM of each FIP and BIP primers, 0.2 μM of each F3 and B3 primers, 0.4 μM of each LB and LF primers, 1.4 mM of each dNTP, 1× Isothermal Amplification Buffer (20 mM Tris–HCl, pH 8.8, 50 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Tween 20), 1 M betaine, 6 mM supplementary $MgSO_4$ and 8 U of *Bst* 2.0 Warm Start DNA polymerase with 2 μL of template purified DNA or heated urine.

Reactions were incubated for 60 min at 63 °C in a heating block and heated at 80 °C for 5–10 min to terminate the reaction. In all field-LAMP trials, several positive (*S. haematobium* genomic DNA) and negative (water) controls were always included. Genomic DNA from adult male and female *S. haematobium*, Egiptian Strain, NR-31682, was obtained from the Schistosomiasis Resource Center for distribution by BEI Resources, NIAID, NIH (https://www.beiresources.org).

When the LAMP reactions were finished, turbidity caused by the accumulation of magnesium pyrophosphate was visually inspected by naked eye after a brief centrifugation. The positive amplification results were also visually detected by adding 2 μL of 1:10 diluted 10 000 concentration SYBR® Green I dye to the reaction tubes. Green fluorescence was clearly observed in positive LAMP reactions, whereas it remained original orange in negative reactions. Testing for LAMP was blinded to both microscopy and microhaematuria results.

After competing LAMP analysis, all samples (purified DNA and previously heated urine) were stored at -20 °C until they were sent in October 2015 to the Center for Research in Tropical Diseases of the University of Salamanca (CIETUS, Salamanca, Spain). The samples were kept frozen whenever possible due to periodic power outages at the Hospital Nossa Senhora da Paz. Additionally, it was not possible to keep the cold chain for shipping the samples to Spain. Once at our reference laboratory, all 172 urine samples were reanalysed by LAMP by the same procedures used in endemic area to assess the reproducibility of the technique. In all laboratory-LAMP trials, several positive (*S. haematobium* genomic DNA) and negative (water) controls were always included. The results were also checked by turbidity and colour change. Besides, 3–5 μL of each LAMP product were used for 2% agarose gel electrophoresis to corroborate the colorimetric results. A GelDoc imaging system (UVItec, UK) was used to observe the band patterns. The positive samples showed a characteristic ladder-like band pattern.

Statistical analyses

Statistical analysis was performed using GraphPad Prism software package (version 6, GraphPad Software, Inc., San Diego, CA, USA; https://www.graphpad.com). Standard statistical tests were used to analyse the groups (microscopy against age, gender and microhaematuria), including chi-square and Fisher's exact test (two-sided). Comparison of LAMP results with those obtained by microscopy and microhaematuria was analysed by McNemar's test for matched pairs. Results were considered statistically significant if P value was < 0.05.

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Results

Microhaematuria and microscopy

Using strip tests, microhaematuria was detected in a total of 83/172 (48.3%) urine samples. *Schistosma haematobium* eggs were detected in a total of 87/172 samples, resulting in an apparent prevalence of infection of 50.6% with no statistically significant associations between age and sex. Besides, 62 of 87 microscopy-positive samples and 21 of the 85 microscopy-negative samples, respectively, tested positive for microhaematuria. The occurrence of microhaematuria was significantly associated with the presence of *S. haematobium* eggs in urine ($P < 0.0001$).

LAMP assay screening under field conditions

The LAMP results obtained after screening the urine samples under field conditions using both purified DNA and heat-treated urine in comparison with microscopy results are summarised in Table 1. Using purified DNA, LAMP-positive results were visually detected by turbidity (LAMP-T+) in 124/172 (72.1%) samples. The number of positive results increased up to 127/172 (73.8%) after adding SYBR® Green I (LAMP-SYBR+). Using non-purified DNA as template for the Rapid-Heat LAMPellet method, we visually detected 98/172 (57%) positive samples by turbidity (RHE-T+). In this case, the number of positive results increased up to 109/172 (63.4%) after adding SYBR® Green I (RHE-SYBR+). Regardless of the quality of the DNA for amplification, in all samples showing turbidity, a change in colour was also obtained when adding SYBR® Green I. The colour change method offered more positive results than turbidity, especially when using non-purified DNA as template in LAMP reactions.

The LAMP results were compared with the urine microscopy findings as the reference standard. Of the 87 microscopy-positive urine samples, 75 (86.2%) resulted LAMP-positive when testing with purified DNA and revealed with SYBR® Green I (LAMP-SYBR+) and 68 (78.2%) when testing with non-purified DNA by the Rapid-Heat LAMPellet method (RHE-SYBR+). Of the 85 microscopy-negative urine samples, 53 (61.2%) resulted LAMP-positive when testing with purified DNA and revealed with SYBR® Green I (LAMP-SYBR+) and 41 (48.2%) when testing non-purified DNA by the Rapid-Heat LAMPellet method (RHE-SYBR+).

The LAMP results (using purified DNA for the amplification and SYBR® Green I to detect amplified products) were also compared with microhaematuria tests. The overlaps of all resulting microscopy, microhaematuria and LAMP assays are shown using Venn diagrams in Figure 1. Up to 56 of the 62 urine samples with both microscopy- and microhaematuria-positive results were LAMP-positive (Figure 1a). It is noteworthy that up to 37 urine samples with both microscopy- and microhaematuria-negative results were also LAMP-positive. On the other hand, 27 urine samples were negative for all three detection tests applied (Figure 1b). MacNemar's test showed a statistical significant relation between LAMP results and microscopy-detected *S. haematobium* infections ($P = 0.0002$) and microhaematuria ($P = 0.0009$), respectively.

Table 1 Results obtained by LAMP assays of urine samples under field in comparison with microscopy. LAMP-T + and LAMP-T −, LAMP-positive and LAMP-negative results obtained by turbidity and using purified DNA. LAMP-SYBR + and LAMP-SYBR -, positive and negative results obtained by adding SYBR Green I and purified DNA: RHE-T + and RHE-T −, positive and negative results obtained by turbidity and using heated urine (the Rapid-Heat-LAMPellet method). RHE-SYBR + and RHE-SYBR −, positive and negative results obtained by adding SYBR Green I and using heated urine (the Rapid-Heat-LAMPellet method)

		Microscopy			
	Total	+ (n = 87)	− (n = 85)	+ (%)	− (%)
LAMP-T +	124	75	49	86.2	57.7
LAMP-T−	48	12	36	13.8	42.3
LAMP-SYBR +	127	75	52	86.2	61.2
LAMP-SYBR −	45	12	33	13.8	38.8
RHE-T +	98	62	36	71.3	42.3
RHE-T −	74	25	49	27.7	57.7
RHE-SYBR +	109	68	41	78.2	48.2
RHE-SYBR −	63	19	44	21.8	51.8

LAMP assay screening at a reference laboratory

Comparison of the LAMP results obtained at a reference laboratory with those obtained under field conditions is shown in Figure 2. When performing the LAMP assays in our laboratory, results were repeated in a total of 124/172 (72.1%) purified DNA samples, counting coincidences in positive (96/127; 75.6%) and negative (28/45; 62.2%) LAMP results (Figure 2a). When non-purified DNA was used as template for the Rapid-Heat LAMPellet method, results were repeated in 85/172 (49.4%), counting coincidences in positive (51/109; 46.8%) and in negative (34/63; 54%) results (Figure 2b). At laboratory, in both LAMP methods, all

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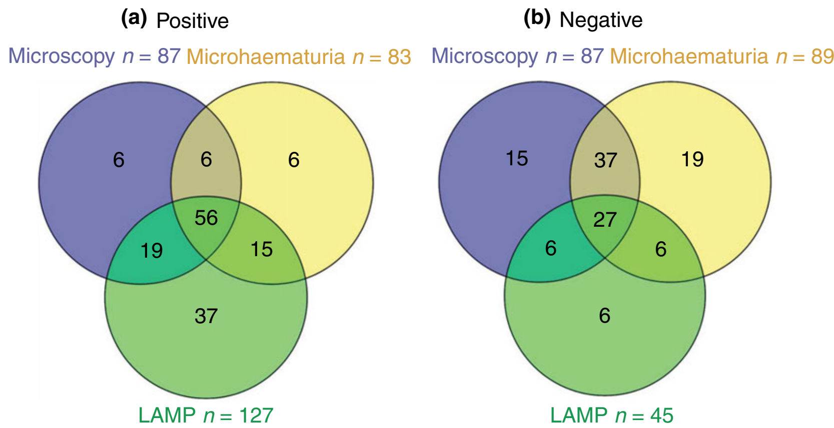

Figure 1 Venn diagram for three-way comparison of microscopy, microhaematuria and LAMP results. Diagram show the results using purified DNA for the amplification and SYBR Green I to detect amplified products. (a) Venn diagrams showing positive results. (b) Venn diagrams showing negative results.

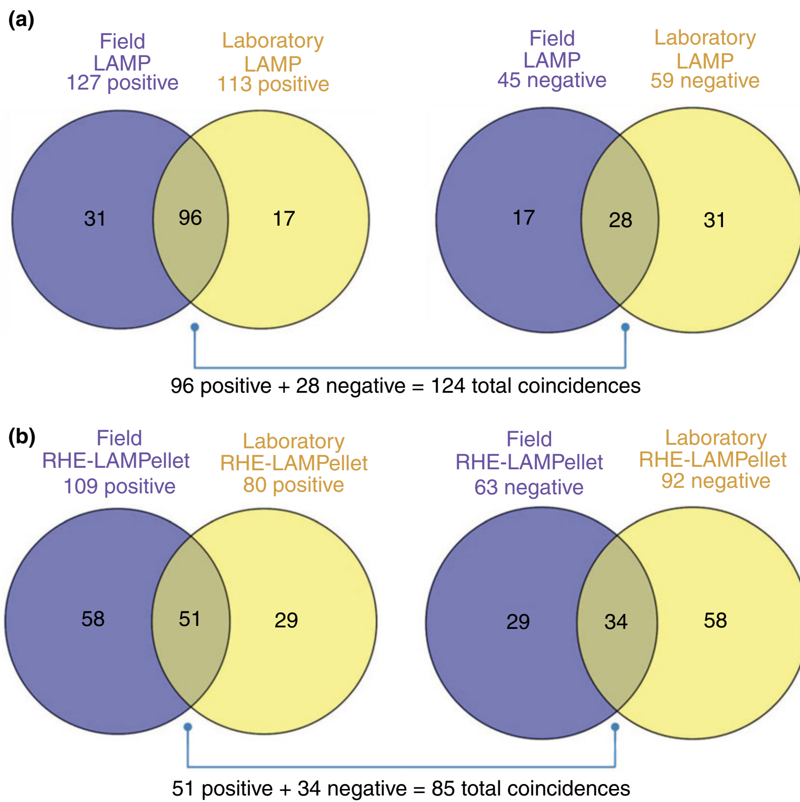

Figure 2 Comparison of the field tests' and laboratory tests' results obtained by LAMP and the Rapid-Heat LAMPellet method. (a) Overlaps between positive and negative LAMP results showing total coincidences. (b) Overlaps between positive and negative RHE-LAMPellet showing total coincidences.

positive results visually detected by turbidity, after adding SYBR® Green I and by electrophoresis, were coincident. As an example, Figure 3 shows the correspondence between colorimetric and agarose results in several samples analysed using purified DNA in the laboratory. Regardless of the quality of the DNA for amplification, the highest number of matches when repeating the LAMP assays –counting positive and negative coincidences – occurred in those samples with a previously microscopy or microhaematuria positive result when testing under field conditions. Data comparing the LAMP assay and the Rapid-Heat LAMPellet method with microscopy and microhaematuria results are collected in Tables S1 and S2.

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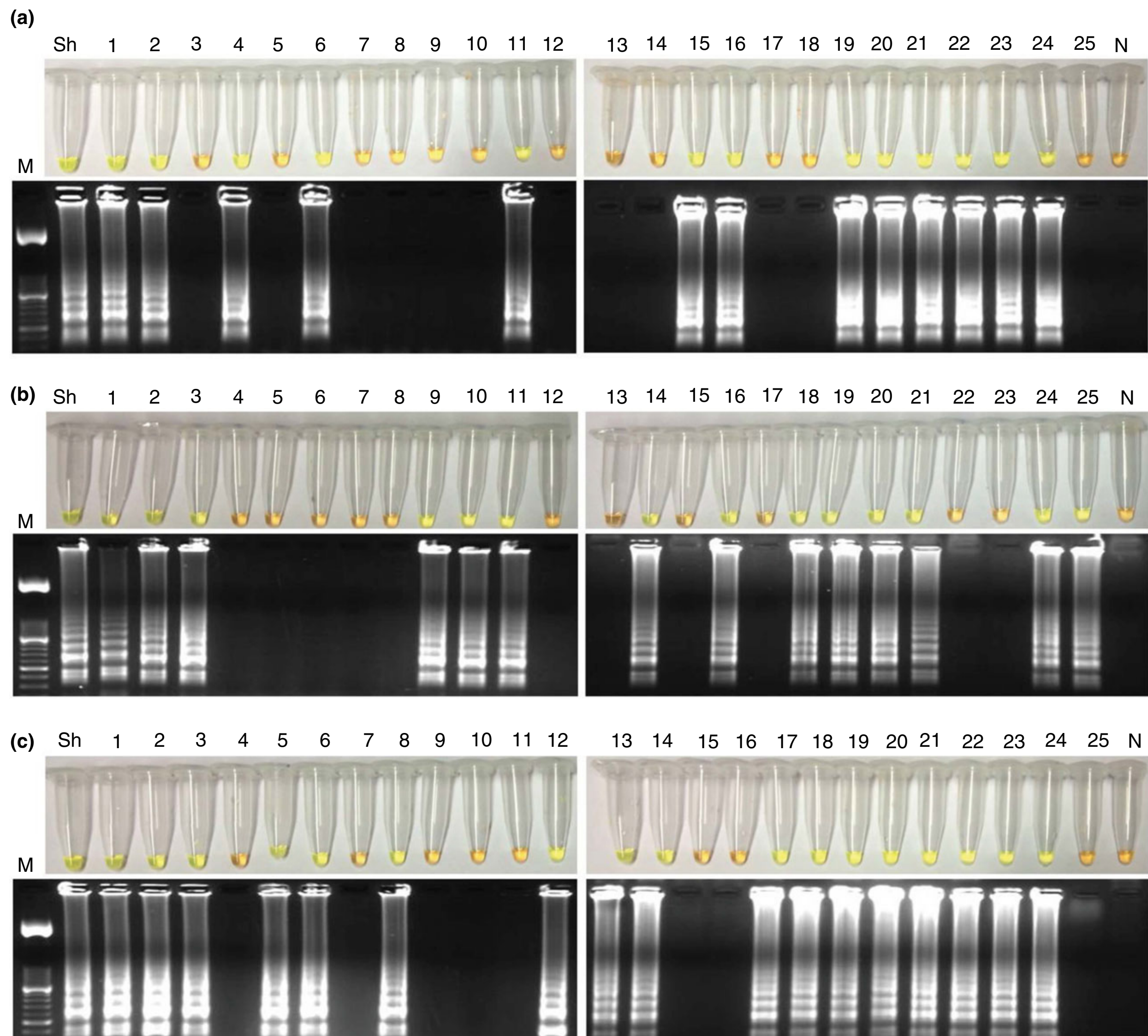

Figure 3 Examination of patients' urine samples by LAMP assay at a reference laboratory. (a) 25 samples from Cubal Sede, (b) 25 samples from Capupa and (C) 25 samples from Yambala. Lanes M, molecular DNA ladder; lanes Sh, positive control (*Schistosoma haematobium* genomic DNA); lanes 1-25, patients' samples; lanes N, negative control (water, no DNA).

Discussion

Direct microscopy observation for eggs of filtered urine remains the 'gold standard' for diagnosis of urogenital schistosomiasis due to its overall accessibility and cost-effectiveness [38]. Reagent strip tests can also be used as a relatively inexpensive option to identify microhaematuria in urine as a diagnostic indicator for *S. haematobium* infection [13]. However, both methods have limitations with regard to sensitivity and the optimal diagnostic test for a urogenital schistosomiasis-endemic region will likely depend on the local intensity of infection [39]. PCR-based methods have been recognised to be good tools to improve diagnostic accuracy for urogenital schistosomiasis, but hardly used for clinical diagnosis in endemic areas because of the technical limitations in low-income countries. Besides, cross-laboratory comparability is difficult and the molecular methods are restricted to a few reference laboratories [14]. Current research is developing alternatives to enable rapid diagnosis with minimal

training including new rapid antigen-based tests, microfiltration technology and DNA-based methods [40]. An enhancement in DNA-based methods is the LAMP technology, which has been advocated as a low-cost genetic analysis tool for resource-poor settings [41]. Therefore, with the aim of improving the molecular diagnostic testing for urogenital schistosomiasis in field conditions, in this study, we evaluated our previously described LAMP assay methodology to detect *S. haematobium* DNA firstly in a low-income schistosomiasis-endemic area and later in a reference laboratory in order to assess the feasibility and reproducibility of the assay.

In this work, the prevalence of *S. haematobium* infection was 50.6% and 48.3% as diagnosed using direct microscopy and urine strip tests, respectively. These values resulted slightly lower than those reported in a recent study in Cubal (61% and 52%, respectively), and no statistically significant association was found in relation to age or sex, thus supporting the idea of a high transmission for *S. haematobium* in Cubal and surroundings [42]. However, in this study, when we tested the urine samples by LAMP assay using purified DNA, the overall prevalence increased significantly reaching 73.8%. As known, diagnosis of *S. haematobium* infection using microscopy may miss cases especially with low burden infection [43], but molecular methods, including PCR [19, 44] and LAMP [34], have been reported to improve sensitivity in diagnosis of schistosomiasis. Our LAMP assay could detect not only DNA from *S. haematobium* eggs but it is also possibly to find cell-free circulating DNA from breakdown products of the parasite, thus increasing sensitivity in the diagnosis. In this way, up to 23.2% more infections were detected by LAMP when comparing to microscopy (73.7% *vs.* 50.6%) in a single urine sample/subject. Additionally, it is important to note that *S. haematobium* DNA was detected by LAMP using a minimal starting processing volume of 1 mL for extraction. Thus, LAMP assay using purified DNA obtained from urine samples appears to be much more sensitive than the urine microscopy findings used commonly as the 'gold standard' test for urogenital schistosomiasis. However, it should be clarified that the use of such a small volume of urine (1 mL) for the extraction of DNA could have been the reason why some parasitological positive samples were negative by LAMP. Possibly, in subsequent studies, it will be necessary to increase the volume of urine samples in order to obtain a greater amount of DNA. Moreover, the LAMP results were easily visualised by turbidity by naked eye in up to 72.1% of the samples tested. This is a great advantage for using in low-income areas compared to other DNA-based molecular methods (such as PCR-based assays) which require equipment and further processing to obtain results.

The total prevalence initially obtained then slightly decreased to 63.4% when crude urine samples were used as templates in the 'Rapid-Heat LAMPellet method' for *S. haematobium* DNA amplification. Even so, this value was higher than that obtained by microscopy (50.6%) and by the urine dipstick tests (48.3%). In addition to our LAMP assay for *S. haematobium*, other studies have shown that LAMP reaction can be performed on crude samples for direct detection of pathogens [45–47], as LAMP is more tolerant to inhibitory substances in biological samples than other DNA-based amplification methods [48]. However, a number of polymerases with strand displacement activity – including the *Bst* polymerase 2.0 Warm Start used here – may be susceptible to various inhibitors in urine samples, thus decreasing sensitivity by up to 10% [49]. In this sense, it would be desirable to carry out additional studies using other commercially available polymerases in order to potentially decrease inhibition and thus improve the sensitivity of the LAMP assay when using crude urine samples. Obviously, a higher sensitivity would be optimal for the Rapid-Heat LAMPellet method in field conditions. Notwithstanding, this molecular approach could be a very useful option for molecular diagnosis of urogenital schistosomiasis in areas where the provision of DNA extraction can be particularly expensive and impracticable. The method could also potentially be applied as a population-based estimator of urogenital schistosomiasis prevalence along with the urine dipstick test for microhaematuria [50]. In this case, the Rapid-Heat LAMPellet method would be species-specific for *S. haematobium* detection, whereas microhaematuria is known to be a non-specific sign of urogenital schistosomiasis [4]. As noted elsewhere, with the use of more sensitive diagnostics, many haeme-positive egg-negative subjects in endemic areas could be detected as *S. haematobium*-infected [13].

Interlaboratory comparisons are needed to determine the repeatability and reproducibility of analytical methods to be standardised [51–53]. In our reference laboratory, we obtained 72.1% of total matches when analysing purified DNA by LAMP. We think that this outcome shows very reasonable agreement between field-based and laboratory-based test results considering the long time spent in inadequate storage of the samples under field conditions. The repeated freezing and thawing as well as the lack of a cold chain for a long time for shipment to our laboratory without the use of any preservative agent for crude urine and DNA samples may have contributed a lot to a potential degradation of DNA, thus decreasing sensitivity in LAMP assay and therefore the number of matching results in amplification. This option would be more relevant in the case of comparison of using crude urine samples as

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template for LAMP assay since only 49.5% of coincidences were obtained at laboratory. It has been reported that urinary DNA deteriorates very quickly as a result of variation in urine specimen storage, handling and preservation [54–56], thus affecting to the performance of successful molecular amplification assays [57]. In this sense, most reports still use LAMP for the detection of DNA already extracted and purified from biological samples. It could be also possible that several other factors could lead to an apparent loss of LAMP efficiency, such as bacterial and/or fungal contamination during the storage of samples. Further studies are needed in order to improve the stability of urinary DNA in fresh urine sediment to be used in LAMP assays without a prior DNA extraction method. This would represent a great promise as an easy and cost-effectiveness molecular diagnostic field test not only for urogenital schistosomiasis, but also for other neglected tropical diseases.

Finally, it is very important to note that regardless of the quality of the DNA used as template for amplification, the highest number of matches between field-LAMP test and laboratory-LAMP test occurred in those samples with a microscopy-positive finding (true infections) and/or microhaematuria-positive finding (very probable infections). This outcome reinforces the reliability of our LAMP assay for *S. haematobium* DNA detection in urine samples.

Conclusions

In our study, we have compared, for the first time, the reproducibility of a LAMP assay testing the same samples in two very different settings: in a resource-poor laboratory in a schistosomiasis-endemic area and in a well-equipped modern laboratory. The ease of use, simplicity and feasibility demonstrated by our LAMP assay in field conditions joined with the acceptable level of reproducibility in a reference laboratory suggest that our LAMP assay designed for specific detection of *S. haematobium* DNA could provide an effective test for molecular diagnosis of urogenital schistosomiasis in disease-endemic remote areas. Nevertheless, further studies are required for additional validation, using a well-preserved urine samples after initial assays in field conditions and multiple repeats on the samples to show robustness of the assay.

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Availability of data and materials

The data set supporting the conclusions of this article is included within the article and additional supporting information files.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

JG, PFS and AMA conceived and designed the experiments. JG, ED, MLARA, ME, AN and ES performed the experiments. JG, EY, PFS and AMA analysed the data. ED, ER, MLARA, ME, CB, FS, ES, MM, JLA and BV contributed reagents/materials/analysis tools. JG, PFS and AMA wrote the manuscript. All authors read and approved the final version of the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Data comparing LAMP assay in field and in the reference laboratory with microscopy and microhaematuria

Table S2. Data comparing the Rapid-Heat LAMPellet method in field and in the reference laboratory with microscopy and microhaematuria

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