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

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	molecular DNA detection by LAMP assay. Urine samples suffered from inadequate storage conditions in a poorly equipped laboratory. Field-LAMP tests were performed with and without prior DNA extraction from urine samples and the results visually detected by turbidity and also by color change. In total, 83/172 (48.30%) were positive for microhaematuria, 87/172 (50.60%) were microscopy positive for S. haematobium eggs detection and, 127/172 (73.83%) showed LAMP positive results for detecting S. haematobium-specific DNA. MacNemar's test showed a statistical significant relation between LAMP results and microscopy-detected S. haematobium infections (p-value 0.0002) and microhaematuria (p-value 0.0009), respectively. When purified DNA samples were analyzed again in a well-equipped laboratory in Spain using the same LAMP methodology, the overall reproducibility achieved 72.10%. Conclusions/Significance: The easily, cost-effectiveness and feasibility demonstrated by our LAMP assay in field conditions joined with the remarkable reproducibility in a reference laboratory, strongly suggests that our LAMP assay could provide an effective test for molecular diagnosis of urogenital schistosomiasis in disease-endemic remote areas.

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

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Abstract

Background: Direct microscopy of filtered urine and reagent strip tests to identify microhaematuria are widely used for diagnosis of urogenital schistosomiasis in poor resource settings. These methods are known to have limitations with regard to sensitivity. In a previous work we designed a LAMP (*loop-mediated isothermal amplification*) assay for the sensible and specific detection of *Schistosoma haematobium* DNA in urine samples. This work aimed to assess the utility of our LAMP for diagnosis of urogenital schistosomiasis in an endemic area in Cubal, Central Angola. In addition, the reproducibility of the assay was evaluated in a reference laboratory for the first time.

Methodology/Principal findings: A total of 172 urine samples from school age children were tested for microhaematuria, microscopic detection of *S. haematobium* eggs and molecular DNA detection by LAMP assay. Urine samples suffered from inadequate storage conditions in a poorly equipped laboratory. Field-LAMP tests were performed with and without prior DNA extraction from urine samples and the results visually detected by turbidity and also by color change. In total, 83/172 (48.30%) were positive for microhaematuria, 87/172 (50.60%) were microscopy positive for *S. haematobium* eggs detection and, 127/172 (73.83%) showed LAMP positive results for detecting *S. haematobium*-

specific DNA. MacNemar's test showed a statistical significant relation between LAMP results and microscopy-detected *S. haematobium* infections (p-value 0.0002) and microhaematuria (p-value 0.0009), respectively. When purified DNA samples were analyzed again in a well-equipped laboratory in Spain using the same LAMP methodology, the overall reproducibility achieved 72.10%.

Conclusions/Significance: The easily, cost-effectiveness and feasibility demonstrated by our LAMP assay in field conditions joined with the remarkable reproducibility in a reference laboratory, strongly suggests that our LAMP assay could provide an effective test for molecular diagnosis of urogenital schistosomiasis in disease-endemic remote areas.

Author Summary

Urogenital schistosomiasis, caused by the trematode flatworm *Schistosoma haematobium*, is the most prevalent form of schistosomiasis in humans, affecting 112 million people mainly in Africa and in the Middle East and emerging in Mediterranean Europe. Chronic infection with *S. haematobium* has been linked with carcinoma of the bladder and increased risk for HIV infection. Direct microscopy of filtered urine remains as the reference for diagnosis and reagent strip tests can also be used to identify microhaematuria in urine as a diagnostic indicator for *S. haematobium* infection. Both methods lack in sensitivity and new molecular diagnostic tools that can improve the sensitivity would be desirable. Currently, LAMP (*loop-mediated isothermal amplification*) technology shows all the characteristics required of a molecular assay with simple operation for potential use in diagnosis of infectious diseases in the field conditions. We recently described a novel LAMP assay to detect *S. haematobium* specific DNA in urine samples with no complicated requirement procedure for DNA extraction for molecular analysis. Here, we use our LAMP assay in a low-income urogenital schistomomiasis-endemic area using purified and non-purified DNA from urine samples for molecular analysis in comparison to microscopy and microhaematuria. Besides, this is the first time that the reproducibility of a LAMP assay was also evaluated in a reference laboratory to compare results. A very reasonable agreement between field-based and laboratory-based test results was obtained considering the long time spent in an inadequate storage of the urine samples under field conditions and shipping. The easily, cost-effectiveness and feasibility demonstrated by our LAMP assay in field conditions joined with the remarkable reproducibility in a reference laboratory could provide an effective molecular tool for diagnosis of urogenital schistosomiasis in disease-endemic remote areas.

Introduction

Urogenital schistosomiasis is a chronic, parasitic disease caused by the blood fluke *Schistosoma haematobium* affecting 112 million people with the highest prevalence living in sub-Saharan Africa, Middle East and emerging 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 kidney damage [4]. In chronic stages of the disease, the deposition of *S. haematobium* eggs eventually allowed 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, association between morbidity and mortality due to *S. haematobium* entails a significant barrier to economic and social development [8].

The microscopic identification of *S. haematobium* excreted 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 acute phase of the disease since the parasites have not yet started to produce eggs. The detection of microhaematuria using reagent strips 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 diagnostic as it is a non-specific sign of urogenital schistosomiasis [4]. Antiboby-based diagnostic tests are useful tools for diagnosing urogenital schistosomiasis in several specific circumstances, but they suffer from many drawbacks and their application is very limited [14,15]. On the other hand, circulating antigen-based detection methods have been developed to diagnose active schistosomiasis with moderate to good reliability in detecting some schistosomes species, but poor accuracy for detecting *S. haematobium* [16].

Several molecular PCR-based methods have been used as alternatives to microscopy and serology for diagnosis urogenital schistosomiasis [17,18]. These methods not only require expensive automated thermal cycler and associated PCR kits but also an acceptable level of training and infrastructure, which does not exist in many low-income countries. A good alternative could be the loop-mediated isothermal amplification (LAMP) assay, a powerful nucleic acid amplification technique which combines rapidity, simplicity, and high specificity [19]. At present, LAMP technology has all the characteristics required of a real-time 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 [22-22]. Additionally, a number of LAMP assays have already been

successfully used for detecting DNA from several schistosomes species, including *S. japonicum, S. mansoni and S. haematobium* [23-32].

The first LAMP assay for high sensitive and specific detection of *S. haematobium* in human urine samples was recently reported by our group [33]. In that work, we demonstrated that simply rapid-heating urinary pellets for good-quality DNA extraction -the Rapid-Heat LAMPellet method- was effective for use in LAMP assays with regard of detecting *S. haematobiun* in a number of patients' urine samples with proven infection with the parasite. Now, the aim of this study was to evaluate that previously described LAMP assay in field diagnosis in a low-income urogenital schistosomiasis-endemic area. In addition, this is the first time that the reproducibility of the LAMP assay was also evaluated in a reference laboratory to compare results.

Methods

Ethics statement

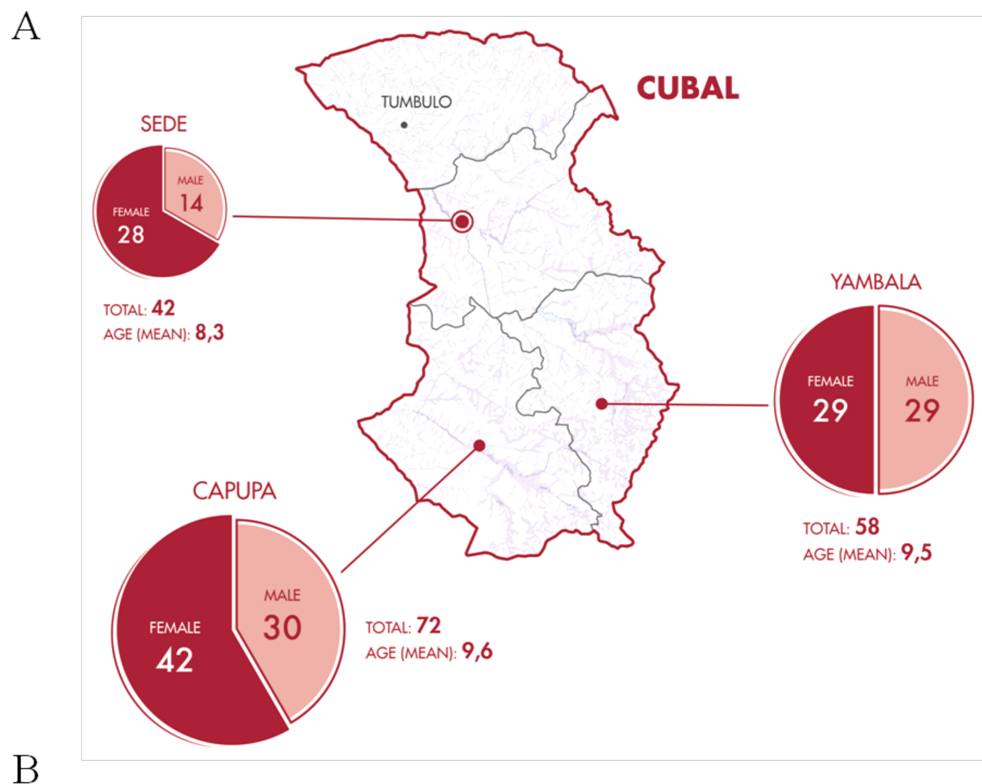
The study was approved by the Ethical Review Board of the Vall d'Hebron University Hospital (Barcelona, Spain) and by the local institutions. Ethical approval was also obtained from the Ethics Committee of the University of Salamanca (protocol approval no. 48531). Participation in the study was voluntary and with prior parental consent. We excluded children whose parents or legal guardians objected to their participation. 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 or children guardian and all students with confirmed infection received appropriate treatment. Procedures were performed in accordance with 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 by 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 [34]. The study population included a total of 252 school-age children ranging 9-10 years old recruited in 10 different schools of Cubal: 2 located in Cubal Sede, 4 in Yambala and 4 in Capupa. Scholars were randomly selected by one of consecutive pairs, in alphabetical order. Participation in the study was voluntary and with prior parental consent. Gender, age, school and community were recorded in a questionnaire.

Urine samples were requested from all children finally included in the study and collected *in situ* during daily classes between 10:00-14:00h for optimum *S. haematobium* egg passage [35]. 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. Unfortunately, several samples (80/252; 31.74%) were excluded from the study due to a microscopy unrealized (54), an incorrect maintenance (16) or lack of information during storing and shipping (10). Thus, a total of 172 urine samples were finally included and tested in the study. Geographical location and demographic information are summarized in Fig 1A.



B

	Microscopy positive; n=87	Microscopy negative; n=85	p value
Age: years, median ± SD	9.62 ± 2.3	8.90 ± 2.4	n.s.
Sex: male/female	39/48	34/51	n.s.
Microhaematuria (%)	62 (71.70)	21 (24.71)	< 0.0001

Fig 1. Map of the study area and population. (A) Geographical location and demographic information. (B) Microscopy and microhaematuria results regarding age and gender.

Urine samples processing and analysis

Microhaematuria and *S. haematobium* eggs detection. First, urine samples were screening for microhaematuria by using reagent strips (COMBI-SCREEN®, Analyticon Biotechnologies, Germany). Then, a volume of 2 mL was reserved and stored at -20ºC for further DNA extraction for molecular analyses. The remaining volume of each urine sample (13 mL, approximately) was processed using the sedimentation by gravity method [36]. 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 obtaining

Aliquots of 2 mL of frozen urine samples were used for DNA obtaining for molecular analysis. After thawing, each aliquot of 2 mL was divided into two new clean microcentrifuge tubes both containing 1 mL. Then, new aliquots of 1 mL were centrifuged at 5000 rpm for 5 min at room temperature (RT) 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. In this way, from each urine sample we finally obtained two aliquots of 100 µL. 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. Later, 2 µL of purified DNA thus obtained were used as template for LAMP amplifications. For the other, the resuspended urinary sediment was heated at 95ºC for 15 min and then briefly spun to pellet the debris. After this, 2 µL of the supernatant were used immediately as template for each LAMP reaction without any purification procedure.

LAMP assay.

Both purified and unpurified DNA obtained from each of the two aliquots of all urine samples were analyzed by conventional LAMP assay and the "Rapid-Heat LAMPellet method", respectively, following the same protocol previously described in Gandasegui et al (2015) [33]. Briefly, 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, 1x 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% Tween20-, 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). Reaction was incubated for 60 min at 63ºC in a heating block and heated at 80ºC for 5-10 min to terminate the reaction.

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 10.000X 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 were blinded to both microscopy and microhaematuria results.

After competing all analysis, samples (purified DNA and crude urine) were stored at -20ºC until they were sent in October 2015 to the Center for Research in

Tropical Diseases of the University of Salamanca (CIETUS, Salamanca, Spain). Urine samples suffered from an inadequate storage conditions in a poorly equipped laboratory. The samples were kept frozen whenever possible due to periodically 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 reanalyzed by LAMP using the same procedures as in endemic area to assess the reproducibility of the technique. Results were also checked by turbidity, color change and, additionally, by electrophoresis to corroborate the colorimetric results.

Statistical analyses

Statistical analyses were 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 analyze the groups, including Chi-square and Fisher's exact test (two-sided). Comparison of LAMP results with those obtained by microscopy and microhematuria were analyzed by McNemar's test for matched pairs. Comparisons were considered significant at a P value < 0.05.

Results

Microhaematuria and microscopy

Using strip tests, microscopic haematuria was detected in a total of 83/172 (48.30%) urine samples. *S. haematobium* eggs were detected in a total of 87/172 samples, resulting in an overall prevalence of infection of 50.58% with no statistically significant associations between age or gender (Fig 1B). Besides, 62 of the 87 microscopy-positive samples and 21 of the 85 microscopy-negative samples, respectively, tested positive for microhaematuria. The occurrence of microhaematuria was significantly ($p<0.0001$) associated with the presence of *S. haematobium* eggs in urine.

LAMP assay screening under field conditions

The LAMP results obtained after screening the urine samples under field conditions using both purified and non-purified DNA in comparison to microscopy results are showed in Fig 2. Using purified DNA (Fig 2.A), LAMP-positive results were visually detected by turbidity (LAMP-T+) in a total of 124/172 (72.10%) samples. The number of positive results increased to 127/172 (73.83%) after adding SYBR® Green I (LAMP-SYBR+). Using non-purified DNA as template for the Rapid-Heat LAMPellet method (Fig 2.B) we visually detected a total of 98/172 (56.97%) positive samples by turbidity (RHE-T+). In this case, the number of positive results increased to 109/172 (63.37%) after adding SYBR® Greeen I (RHE-SYBR+). Regardless of the quality of the DNA for amplification, in all samples showing a white pellet a change in color was also obtained when adding SYBR®

Green I. The color-change method offered more positive results than turbidity, especially when using non-purified DNA as template in LAMP reactions.

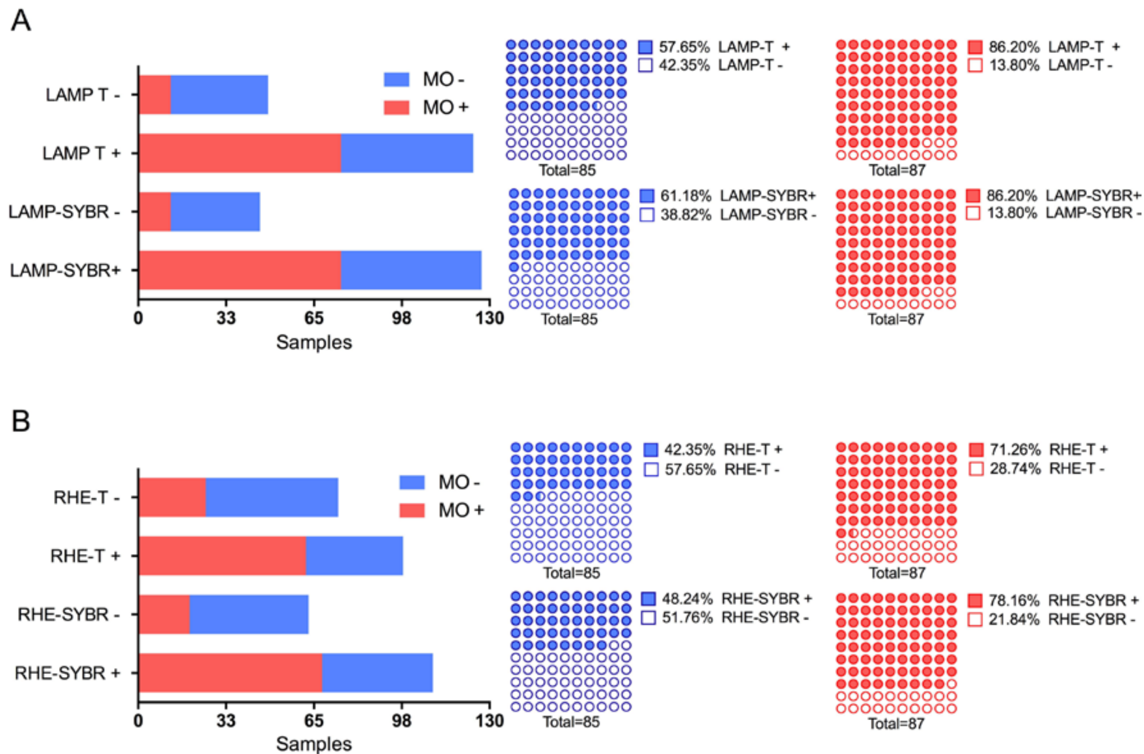


Fig 2. Results obtained by LAMP assays of urine samples under field conditions using purified and non-purified DNA in comparison to microscopy. (A) LAMP assay using purified DNA. Blue and red color show microscopy negative (MO-) and positive (MO+) samples for *S. haematobium*. Graphics bars show the number of LAMP negative (LAMP T-) and positive (LAMP T+) results obtained by turbidity and negative (LAMP-SYBR-) and positive (LAMP-SYBR+) adding SYBR® Green, respectively. Circles show the percentage of LAMP positive and negative samples in comparison to microscopy positive and negative samples. (B) LAMP assay using non-purified DNA: Rapid-Heat LAMPellet method (RHE). Blue and red color show microscopy negative (MO-) and positive (MO+) samples for *S. haematobium*. Graphics bars show the number of LAMP negative (RHE-T-) and positive (RHE-T+) results obtained by turbidity and negative (RHE-SYBR-) and positive (RHE-SYBR+) adding SYBR® Green, respectively. Circles show the percentage of LAMP positive and negative samples in comparison to microscopy positive and negative samples.

The LAMP results were compared with the urine microscopy findings as the reference standard. Of the total of 87 microscopy-positive urine samples, 75 (86.20%) resulted LAMP-positive when testing with purified DNA and revealed with SYBR® Green I (LAMP-SYBR+) and 68 (78.16%) when testing with non-purified DNA by the Rapid-Heat LAMPellet method (RHE-SYBR+). Of the total of 85 microscopy-negative urine samples, 53 (61.18%) resulted LAMP-positive when testing with purified DNA and revealed with SYBR® Green I (LAMP-SYBR+) and 41

(48.24%) when testing non-purified DNA by the Rapid-Heat LAMPellet method (RHE-SYBR+).

The LAMP results were also compared with microhaematuria tests. The overlaps of all resulting microscopy, microhaematuria and LAMP assays are shown using Venn diagrams in Fig 3. Up to 56 of the 62 urine samples with both microscopy and microhematuria positive results were LAMP-positive (Fig 3.A). It is noteworthy that up 37 urine samples with both microscopy and microhematuria negative results were also LAMP-positive. On the other hand, a total of 27 urine samples were negative for all three detection tests applied (Fig 3.B). MacNemar's test showed a statistical significant relation between LAMP results and microscopy-detected *S. haematobium* infections (p-value 0.0002) and microhematuria (p-value 0.0009), respectively.

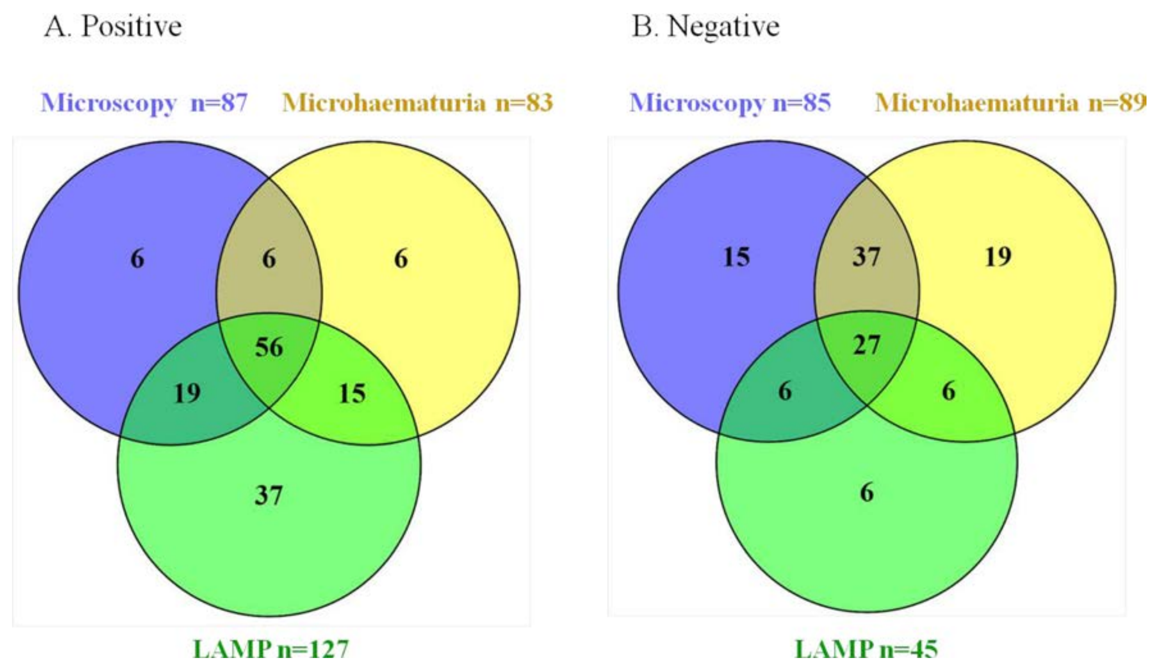


Fig 3. Venn diagram for 3-way comparison of microscopy, microhaematuria and LAMP results. (A) Venn diagrams showing positive results. (B) Venn diagrams showing negative results.

LAMP assay screening at a reference laboratory

Comparison of the LAMP results obtained at a reference laboratory with those previously obtained under field conditions is shown in Fig 4. When performing the LAMP assays in our laboratory, results were repeated in a total of 124/172 (72.09%) purified DNA samples, counting coincidences in positive (96/127; 75.59%) and negative (28/45; 62.22%) results (Fig 4A). When non-purified DNA was used as template for the Rapid Heat-LAMPellet method, results were repeated in 85/172 (49.41%), counting coincidences in positive (51/109; 46.78%) and in

negative (34/63; 53.96%) results (Fig 4B). At laboratory, in both LAMP methods, all positive results visually detected by turbidity, after adding SYBR® Green I and also by electrophoresis were coincident. As an example, Fig 5 shows the correspondence between colorimetric and agarose results in a number of samples analyzed 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 S1 Table and S2 Table, respectively.

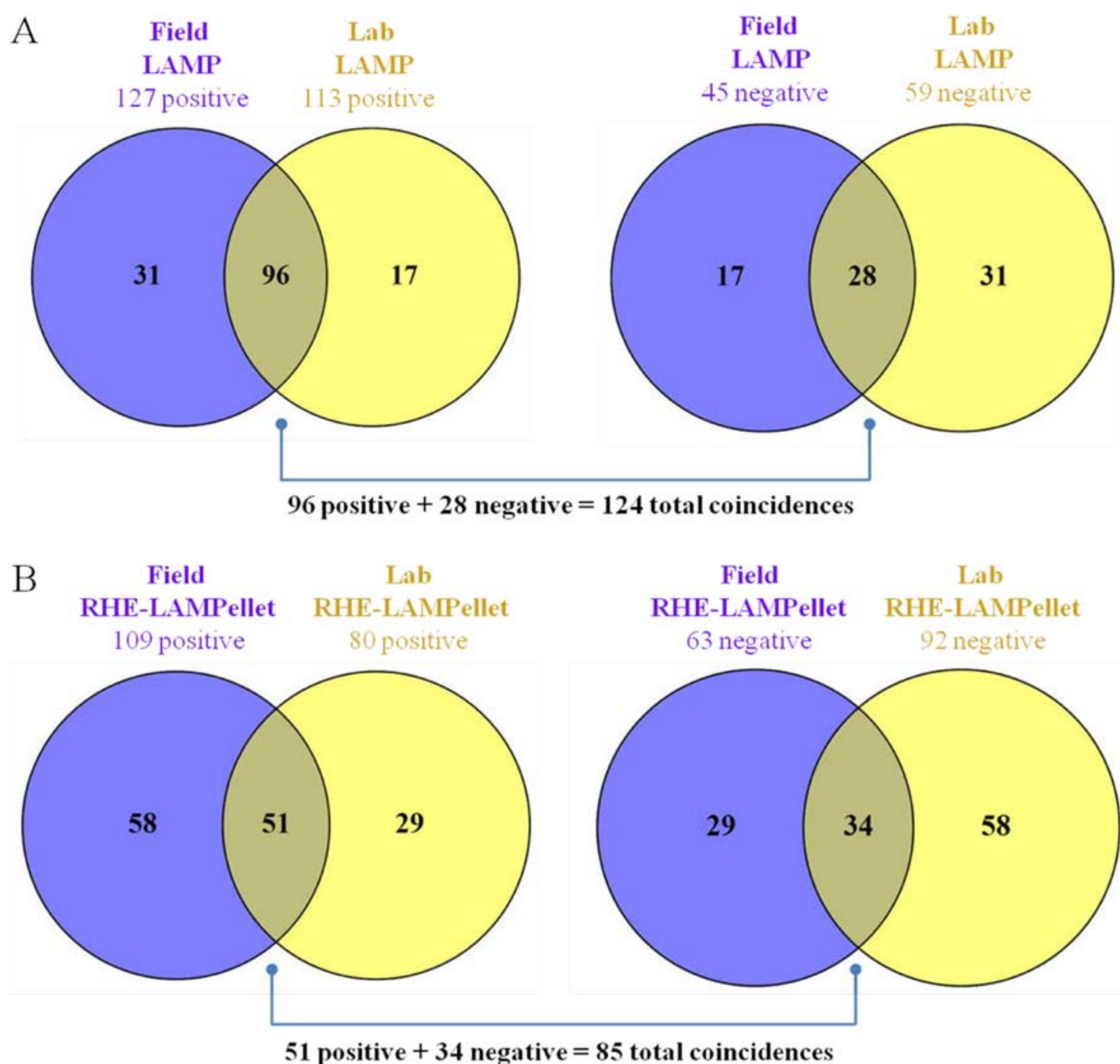


Fig 4. Comparison of the field-tests and lab-tests results obtained by LAMP and the Rapid-Heat LAMPellet method. (A) Overlaps between positive and negative LAMP results showing total coincidences. (B) Overlaps between negative positive and negative RHE-LAMPellet showing total coincidences.

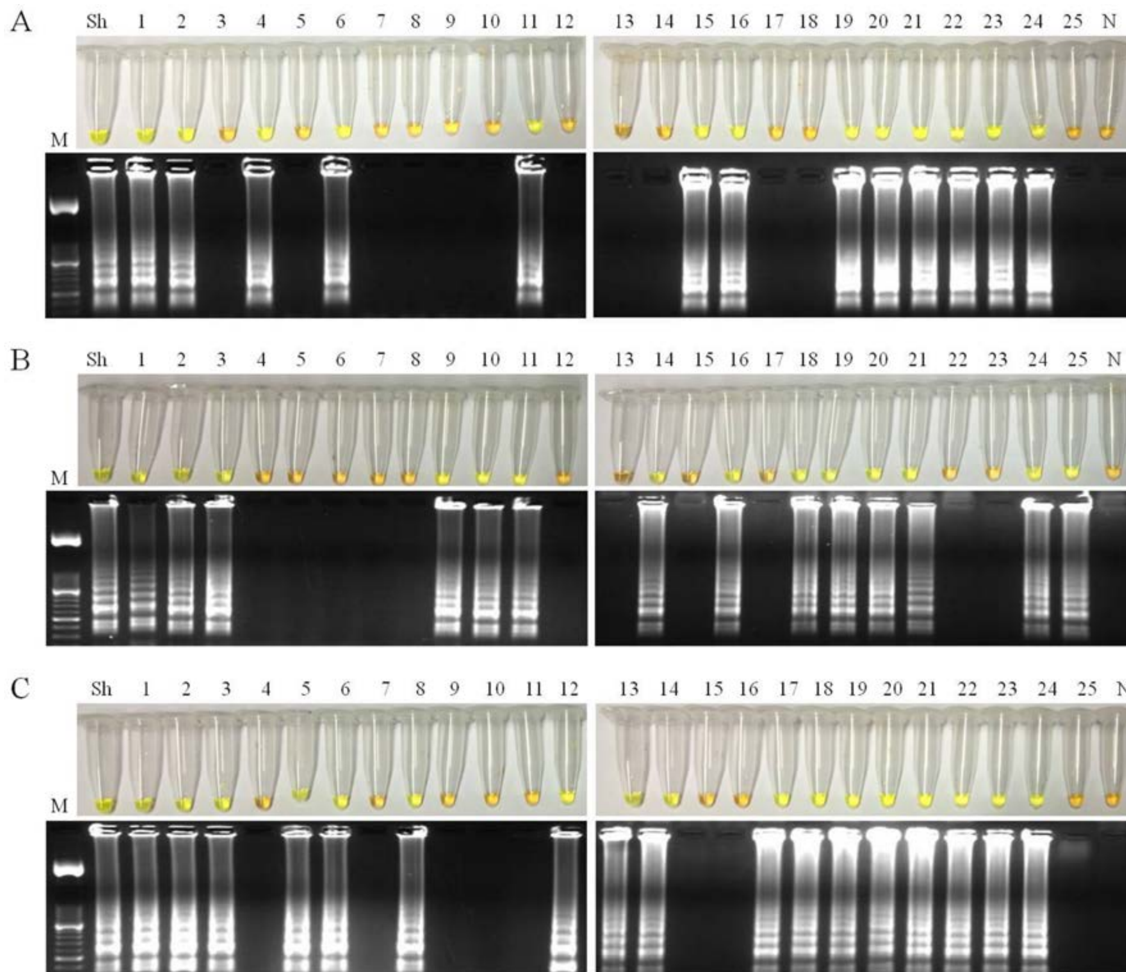


Fig 5. Examination of patients' urine samples by LAMP assay using purified DNA as template for amplification at a reference laboratory. (A) Twenty-five samples from Cubal Sede, (B) twenty-five samples from Capupa and (C) twenty-five samples from Yambala. Lanes M, molecular DNA ladder; lanes Sh, positive control (*S. haematobium* genomic DNA); lanes 1-25, patients' samples; lanes N, negativo control (water, no DNA).

Discussion

Direct microscopy of filtered urine remains as the 'gold standard' for diagnosis of urogenital schistosomiasis due to its overall accessibility and cost-effectiveness [37]. 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 an urogenital schistosomiasis-endemic region will likely depend on the local intensity of infection [38]. PCR-based methods have been proven 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 [39]. 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, in this study, with the aim of improving the molecular diagnostic testing for urogenital schistosomiasis in field conditions, we evaluated our previously described LAMP assay 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 50.58% and 48.30% 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 also found in relation to age or gender, thus supporting the idea of a high transmission for *S. haematobium* in Cubal and surroundings [42]. However, in our study, when we tested the urine samples by LAMP assay using purified DNA, the overall prevalence increased significantly reaching 73.83%. As known, diagnosis of *S. haematobium* infection using microscopy may miss cases especially during light infection [43] but molecular methods, including PCR [44, 45] and LAMP [46], have been reported to improve sensitivity in diagnosis of schistosomiasis. Our LAMP assay could detect not only DNA from *S. haematobium* eggs but also transrenal DNA from breakdown products of the parasite thus increasing sensitivity in the diagnosis. In this way, up to 23.25% more infections were detected by LAMP when comparing to microscopy (73.73% *vs.* 50.58%) 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 100 µL for extraction instead 13 mL used for microscopy. 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. Moreover, the LAMP results were easily visualized by turbidity by the naked eye up to 72.10% of the samples tested. This is a great advantage in low-income areas compared to other DNA-based molecular methods.

The total prevalence initially obtained subsequently slightly decreased to 63.37% when crude urine samples were used as template in the 'Rapid-Heat LAMPellet method' for *S. haematobium* DNA amplification and color change displayed. Even so, this value was higher to that obtained by microscopy (50.58%) and also by the urine dipstick tests (48.30%). In addition to our LAMP assay for *S. haematobium*, other several studies have shown that the LAMP reaction can be performed on crude samples for direct detection of pathogens [47-49], since LAMP is more tolerant to inhibitory substances in biological samples than other DNA-based

amplification methods [50]. However, various polymerases with strand displacement activity –as the *Bst* polymerase 2.0 Warm Start used here- may be susceptible to various inhibitors in urine samples, thus slightly decreasing up 10% in sensitivity [51]. In this sense, it would be desirable to carry out additional studies using other commercially available polymerases in order to potentially increase inhibitor resistance and thus improving 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 in molecular diagnostic of urogenital schistosomiasis in areas where the provision of DNA extraction can be particularly expensive and impracticable. The method could be also potentially applied as a population-based estimator of urogenital schistosomiasis prevalence as well as urine dipstick test for microhaematuria [52]. In this case, the Rapid-Heat LAMPellet method would be species-specific for *S. haematobium* detection whereas microhaematuria is kwon to be a non-specific sign of urogenital schistosomiasis [4]. As noted elsewhere, with the use of more sensitive diagnostics many heme-positive egg-negative subjects in endemic areas could be detected as *S. haematobium*-infected [13].

In the reference laboratory study, we obtained 72.09% of total matches when analyzing again 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 an 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 may have decisively contributed 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 template for LAMP assay since only 49.51% of coincidences were obtained at lab. It has been reported that urinary DNA deteriorates very quickly as a result of variation in urine specimen storage, handling and preservation [53-55] thus affecting to the performance of successful molecular amplification assays [56]. 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 (truly infections) and/or microhaematuria positive finding (very probable infections). This outcome reinforce the reliability of our LAMP assay for *S. haematobium* DNA detection in urine samples.

Interlaboratory comparisons are needed to determine the repeatability and reproducibility of analytical methods to be standardized [57-59]. 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 easily, cost-effectiveness and feasibility demonstrated by our LAMP assay in field conditions joined with the remarkable reproducibility in a reference laboratory, strongly suggests 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.

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S1 Table. Data comparing LAMP assay with microscopy and microhaematuria.

	LAMP +			LAMP -			
Tests	Field	Lab	Coincidences (%)	Field	Lab	Coincidences (%)	Total coincidences (%)
MO + n=87	75	68	61/75 (81.33)	12	19	5/12 (41.66)	66/87 (75.86)
MO – n=85	52	44	34/52 (65.38)	33	41	23/33 (69.69)	57/85 (67.06)
MH + n=83	71	66	62/71 (87.32)	12	17	8/12 (66.67)	70/83 (84.34)
MH – n=89	56	46	33/56 (58.93)	33	43	20/33 (60.60)	53/89 (59.55)
Total n=172	127	113	96/127 (75.59)	45	59	28/45 (62.22)	124/172 (72.09)

MO+, MO- : microscopy positive and negative
MH+, MH-: microhaematuria positive and negative

S2 Table. Data comparing Rapid-Heat LAMPellet method with microscopy and microhaematuria.

	RHE-LAMPellet +			RHE-LAMPellet -			
Tests	Field	Lab	Coincidences (%)	Field	Lab	Coincidences (%)	Total coincidences (%)
MO + n=87	68	48	37/68 (54.41)	19	39	8/19 (42.10)	45/87 (51.72)
MO – n=85	41	32	14/41 (34.14)	44	53	26/44 (59.09)	40/85 (47.05)
MH + n=83	61	48	38/61 (62.29)	22	35	12/22 (54.54)	50/83 (62.24)
MH – n=89	48	32	13/48 (27.08)	41	57	22/41 (53.65)	35/89 (39.32)
Total n=172	109	80	51/109 (46.78)	63	92	34/63 (53.96)	85/172 (49.41)

MO+, MO- : microscopy positive and negative
MH+, MH-: microhaematuria positive and negative