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Development of a Loop-Mediated Isothermal Amplification Method for Detection of *Histoplasma capsulatum* DNA in Clinical Samples

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Improved methods for the detection of *Histoplasma capsulatum* are needed in regions with limited resources in which the organism is endemic, where delayed diagnosis of progressive disseminated histoplasmosis (PDH) results in high mortality rates. We have investigated the use of a loop-mediated isothermal amplification (LAMP) assay to facilitate rapid inexpensive molecular diagnosis of this disease. Primers for LAMP were designed to amplify the *Hcp100* locus of *H. capsulatum*. The sensitivity and limit of detection were evaluated using DNA extracted from 91 clinical isolates of known geographic subspecies, while the assay specificity was determined using DNA extracted from 50 other fungi and *Mycobacterium tuberculosis*. Urine specimens ($n = 6$) collected from HIV-positive individuals with culture- and antigen-proven histoplasmosis were evaluated using the LAMP assay. Specimens from healthy persons ($n = 10$) without evidence of histoplasmosis were used as assay controls. The *Hcp100* LAMP assay was 100% sensitive and specific when tested with DNA extracted from culture isolates. The median limit of detection was ≤ 6 genomes (range, 1 to 300 genomes) for all except one geographic subspecies. The LAMP assay detected *Hcp100* in 67% of antigen-positive urine specimens (4/6 specimens), and results were negative for *Hcp100* in all healthy control urine specimens. We have shown that the *Hcp100* LAMP assay is a rapid affordable assay that can be used to expedite culture confirmation of *H. capsulatum* in regions in which PDH is endemic. Further, our results indicate proof of the concept that the assay can be used to detect *Histoplasma* DNA in urine. Further evaluation of this assay using body fluid samples from a larger patient population is warranted.

Histoplasma capsulatum is a dimorphic fungus that causes histoplasmosis. In immunocompromised persons, *H. capsulatum* can disseminate throughout the body, causing progressive disseminated histoplasmosis (PDH), which is characterized by fever, weight loss, and hepatosplenomegaly. Without early diagnosis and antifungal intervention, PDH can cause death.

Timely detection of PDH is problematic in resource-challenged countries, since few rapid assays exist for this disease (1) and its symptoms are vague and often confused with those of mycobacterial or leishmanial infections (2, 3). Many laboratories in resource-limited areas in which the disease is endemic rely on sterile-site cultures for diagnosis of PDH; however, *H. capsulatum* grows slowly and may take several weeks for identification in cultures. The AccuProbe *H. capsulatum* culture identification test (Gen-Probe) can be used for rapid molecular identification of *H. capsulatum* in cultures, but this test is expensive and is not readily available in developing countries. Several additional molecular assays for detection of *H. capsulatum* have been developed (1, 4, 5), but none has been subjected to large-scale interlaboratory evaluation. These assays rely on PCR methodology and require expensive reagents and equipment, which may be unsustainable in laboratories with limited funding.

Here we describe the development of a loop-mediated isothermal amplification (LAMP) assay for histoplasmosis, which provides an affordable method of molecular identification that can be performed and interpreted without costly equipment in resource-challenged regions. Briefly, LAMP is a nucleic acid amplification technique that utilizes a polymerase with helicase activity, *Bst* from *Bacillus stearothermophilus*. The helicase activity allows for amplification of DNA at a constant temperature and is facilitated by four primers, 2 with cDNA, that form stem-loop DNA structures. Once formed, the stem-loop structures become the tem-

plate DNA for further amplification, which occurs very rapidly (6).

Nucleic acid amplification via LAMP has several cost advantages over PCR. First, *Bst* polymerase is less expensive and more robust than *Taq* (7–9). Further, LAMP requires no thermal cycling equipment, since the assay is performed at a single temperature, allowing the use of either a heat block or a water bath to achieve nucleic acid amplification. Reactions are carried out in single tubes, and results can be visualized under UV light. In order to facilitate rapid inexpensive molecular diagnosis of PDH, we developed a LAMP assay targeting the single-copy gene *Hcp100*. *Hcp100* is a member of the p100 gene family and is overexpressed in *H. capsulatum* during macrophage invasion (10). Unlike many multicopy housekeeping genes, *Hcp100* shows little sequence identity with the DNA of related organisms and is not prone to false hybridization that may lead to cross-reactivity.

MATERIALS AND METHODS

***H. capsulatum* isolates.** *H. capsulatum* isolates ($n = 91$) used in this study were cultured from frozen mycelial stocks provided by Roche Molecular

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Systems (Pleasanton, CA). Mycelia were grown on brain heart infusion (BHI) agar slants and subcultured three times to ensure optimal growth and purity prior to DNA extraction. All isolates were previously identified with respect to their geographic subspecies by multilocus sequence typing (MLST) and phylogenetic analysis (11), as described by Theodoro et al. (12). The geographic subspecies of study isolates were as follows: four North American 1 (NA_m 1), 65 North American 2 (NA_m 2), 11 Latin American A (LA_m A), five Latin American B (LA_m B), two lineage H81, and one each African, Netherlands, lineage H66, and lineage H68.

Fungal DNA extraction. Genomic DNA was extracted using a Qiagen DNeasy tissue kit (Qiagen, Valencia, CA), with several modifications to the manufacturer's instructions. Briefly, a portion of the fungal mat was transferred to 5-ml polypropylene tubes containing 800 μ l Qiagen ATL buffer and 60 U of proteinase K and was homogenized inside a biological safety cabinet using an Omni tissue homogenizer (Omni International, Kennesaw, GA) at slow speed for 30 s and then at high speed for 30 s, using a clean probe for each isolate. Homogenates were capped, incubated at 55°C for 1 h with frequent vortex mixing, and then cooled to room temperature (RT). For each homogenate, RNase A (Sigma-Aldrich Corp., St. Louis, MO) was added to a final concentration of 1 mg/ml and the mixture was incubated for 5 min at RT, followed by the addition of 900 μ l Qiagen buffer AL and vortex mixing. Homogenates were incubated at 70°C for 10 min, transferred to 1.7-ml microcentrifuge tubes, and centrifuged at 15,000 \times g for 10 min. Clear supernatants (1 ml each) were transferred to clean microcentrifuge tubes, and 500 μ l of 200-proof genomic-grade ethanol (Sigma-Aldrich Corp.) was added to each tube. The suspensions were vortex mixed and transferred to Qiagen DNeasy columns; the manufacturer's instructions were followed throughout the remainder of the procedure, except that DNA was eluted with 0.01 M Tris. DNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Archived fungal DNA samples used as controls were buffer exchanged into 0.01 M Tris using the Qiagen protocol for cleanup of genomic DNA (13), in order to eliminate EDTA and other additives known to interfere with subsequent applications.

PCR and sequencing of the *Hcp100* genetic locus. Portions of the *H. capsulatum Hcp100* gene were PCR amplified using Hc I (5'-GCGTTCC GAGCCTTCCACCTCAAC-3') and Hc II (5'-ATGTCCCATCGGGCGC CGTGAGT-3') primers, as described previously (14). Each 25- μ l reaction mixture contained 0.2 μ M each primer, 0.25 mM MgCl₂, 0.2 mM each deoxynucleoside triphosphate (dNTP), and 0.625 U *Taq* DNA polymerase in a buffer of 10 mM Tris (pH 8.3), 50 mM KCl (Roche Carolina Inc., Florence, SC). Thermal cycling was performed in MicroAmp 96-well optical reaction plates, using a GeneAmp PCR system 9700 (Applied Biosystems, Inc., Foster City, CA), as follows: 1 cycle of 94°C for 5 min; 40 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; and a final cycle of 72°C for 5 min. The resulting amplicons were sized on 1.75% agarose gels, visualized with ethidium bromide under UV light, and cleaned using Exo-SAP-IT (USB Corp., Cleveland, OH), according to the manufacturer's instructions. Amplicons were sequenced using the amplification primers Hc I and Hc IV (5'-AGGAGAGAACTGTATCGGTGGC TT-3') (14) and a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc.), and reactions were analyzed with a 3730 DNA analyzer (Applied Biosystems, Inc.).

Comparative sequence analysis and LAMP primer design. Consensus sequences among 14 isolates from geographically and genetically diverse clades were generated from raw data using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, MI) and were aligned using MUSCLE (15) in the MEGA 5.05 software package (16). Primers for LAMP were designed on the basis of conserved regions of the reverse complement of *Hcp100* using Primer Explorer 4, a Web-based, open-use, primer design program (Eiken Chemical Co., Ltd., Tokyo, Japan) (<http://primerexplorer.jp/elamp4.0.0/index.html>). Primers used for LAMP were forward inner primer (FIP), backward inner primer (BIP), forward outer primer (F3), and backward outer primer (B3) (Table 1).

TABLE 1 Primers used for LAMP of the *Hcp100* genetic locus of *H. capsulatum*

Primer	Sequence (5' to 3')
FIP	TCCCCGCGTCTCCCGAATACCGATCCAATGTCGTTCCACC
BIP	TCTGCACGGAAAACTGCGGCCTACGGCAACTCCGAAACC
F3	GTAGTCGACGTTTCGCAACT
B3	GCCGACGTCGTTTACATCG

Clinical specimens. Six urine specimens (one specimen per person) from HIV-infected persons with symptoms consistent with PDH, positive *Histoplasma* urine antigen test results, and culture-confirmed *H. capsulatum* infections were collected between 2004 and 2009 at the Clinica Familiar Luis Angel Garcia (Guatemala City, Guatemala) (17), under conditions reviewed and approved by the internal review boards of the Centers for Disease Control and Prevention and the Universidad del Valle de Guatemala. Ten urine specimens were collected from healthy persons who had no indication of histoplasmosis. Two "mock-positive" samples were prepared from different pools of histoplasmosis-negative urine samples, collected from healthy persons, by spiking with 1 ng *H. capsulatum* DNA prior to extraction.

Urine DNA extraction and PCR. A QIAmp circulating nucleic acid kit (Qiagen) was used to extract DNA from urine specimens, according to the manufacturer's instructions. Briefly, 4 ml urine was centrifuged at 16,000 \times g for 10 min, and the pellet was resuspended in 0.01 M phosphate-buffered saline (PBS) (pH 7.2). Both the supernatant and the resuspended pellets were subjected to DNA extraction using lysis buffers ATL and ACB, according to the manufacturer's instructions. A vacuum manifold designed for DNA extraction (Promega, Madison, WI) was attached to a rotary vane vacuum pump (Gast, Benton Harbor, MI) with a filter trap, and Qiagen Mini columns were attached to the vacuum using adapters provided in the kit. After lysis, the urine specimens were pulled through the columns with the vacuum pump at a pressure of $-85,000$ pascals, bound to the resin columns, and washed. Nucleic acids were eluted in 30 μ l of 0.01 M Tris-HCl by centrifugation and were quantified using a NanoDrop ND-1000 spectrophotometer.

All urine specimen DNA was subjected to PCR using positive-control primers Beta 2 and Beta 3, which amplify portions of the human β -globin (BG) gene (Beta 2 [G1], 5'-GAAGAGCCAAGGACAGGTAC; Beta 3 [G2], 5'-CAACTTCATCCAGTTCACC) (18), and *H. capsulatum* Hc I/Hc IV primers specific for the *Hcp100* genetic locus (14). The resulting amplicons were visualized on agarose gels as described above.

LAMP method. Reaction mixtures were prepared according to the manufacturer's specifications, using a Loopamp DNA amplification kit (Eiken Chemical Co., Ltd.). Each 25- μ l reaction mixture contained 12.5 μ l 2 \times reaction buffer [40 mM Tris-HCl (pH 8.8), 20 mM KCl, 16 mM MgSO₄, 20 mM (NH₄)₂SO₄, 1.6 M betaine, 0.2% Tween 20, 2.8 mM each dNTP], 40 pmol FIP, 40 pmol BIP, 10 pmol F3, 10 pmol B3, 1 μ l *Bst* polymerase (Loopamp fluorescent detection reagent; Eiken Chemical Co., Ltd.), 2 μ l of DNA, and 1 μ l calcein-MnCl₂ dye. Reaction mixtures were incubated at 63°C for 1.5 h, and the reactions were stopped at 95°C for 2 min. Amplification of *H. capsulatum* DNA was detected as calcein fluorescence directly visualized over a UV light box, in comparison with a lack of fluorescence in control reactions to which either non-*H. capsulatum* DNA or no DNA template was added (19).

LAMP assay validation. All *H. capsulatum* clinical isolates ($n = 91$) were assayed using the *Hcp100* LAMP assay to determine its sensitivity. To define the limit of detection (LOD), DNA from 39 *H. capsulatum* isolates, including at least one representative of each geographic subspecies, was diluted 10-fold (from 1 ng/ml to 10 fg/ml) and assayed using LAMP. To test for potential cross-reactivity, archived DNA extracted from clinical fungal isolates maintained by the Mycotic Diseases Branch ($n = 50$) (Table 2) was assayed at concentrations of ≥ 2 ng/ μ l. Tenfold dilutions (50 ng/ μ l to 5 fg/ μ l) of *Mycobacterium tuberculosis* (ATCC 25177D-5) and

TABLE 2 Fungal isolates ($n = 50$) tested in the LAMP assay for detection of *H. capsulatum*

Isolate	Genus and species
CDC B8815	<i>Lichtheimia corymbifera</i>
CDC B8816	<i>Lichtheimia corymbifera</i>
CDC B7759	<i>Apophysomyces elegans</i>
CDC B7460	<i>Apophysomyces elegans</i>
NRRL 485	<i>Aspergillus flavus</i>
IFI 03 0139	<i>Aspergillus flavus</i>
CDC B6077	<i>Aspergillus fumigatus</i>
SRH 21	<i>Aspergillus fumigatus</i>
ATCC 1015	<i>Aspergillus niger</i>
IBT 14590	<i>Aspergillus terreus</i>
UC141	<i>Aspergillus terreus</i>
CDC B8899	<i>Aspergillus versicolor</i>
CDC B8900	<i>Aspergillus versicolor</i>
UC24457	<i>Blastomyces dermatitidis</i>
CDC B3591	<i>Blastomyces dermatitidis</i>
CAS 4016	<i>Candida albicans</i>
CAS 4011	<i>Candida dubliniensis</i>
CAS 3998	<i>Candida glabrata</i>
CAS 4015	<i>Candida krusei</i>
CAS 4031	<i>Candida lusitanae</i>
CAS 4000	<i>Candida parapsilosis</i>
CAS 4012	<i>Candida tropicalis</i>
2010-18016	<i>Coccidioides immitis</i>
2011-02345	<i>Coccidioides posadasii</i>
CDC B9302	<i>Cryptococcus gattii</i>
CDC B7233	<i>Cryptococcus gattii</i>
CDC B9031	<i>Cryptococcus neoformans</i>
CDC B9039	<i>Cryptococcus neoformans</i>
CDC B8703	<i>Fusarium incarnatum-equiseti</i>
CDC B8704	<i>Fusarium incarnatum-equiseti</i>
CDC B8723	<i>Fusarium oxysporum</i>
CDC B8724	<i>Fusarium oxysporum</i>
CDC B8601	<i>Fusarium solani</i>
CDC B8602	<i>Fusarium solani</i>
CDC B2699	<i>Microsporum equinum</i>
CDC B7558	<i>Mucor circinelloides</i>
CDC B7402	<i>Mucor indicus</i>
CDC B9043	<i>Penicillium</i> sp., not <i>marneffei</i>
CDD B9044	<i>Penicillium</i> spp., not <i>marneffei</i>
UC202	<i>Pneumocystis jirovecii</i>
UC246	<i>Pneumocystis jirovecii</i>
UC267	<i>Pneumocystis</i> spp.
CDC B7658	<i>Rhizopus oryzae</i>
CDC B7662	<i>Rhizopus oryzae</i>
CDC B3604	<i>Sporothrix schenckii</i>
CDC B3759	<i>Sporothrix schenckii</i>
CDC B3772	<i>Sporothrix schenckii</i>
CDC B3909	<i>Trichophyton mentagrophytes</i>
CDC B9131	<i>Trichosporon asahii</i>
CDC B9132	<i>Trichosporon asahii</i>

human (catalog no. G304A; Promega, Madison, WI) genomic DNA were also assayed. All DNA and clinical specimen extracts were tested in duplicate. Two negative controls (human DNA and no DNA template) and at least one positive control were included in each LAMP procedure, and assays were repeated twice, to ensure reproducibility.

RESULTS

LAMP assay validity with *Histoplasma* isolates. The *Hcp100* LAMP assay was able to detect DNA of all geographically diverse

TABLE 3 LOD of the LAMP assay for detection of isolates of different geographic subspecies ($n = 39$)

Geographic subspecies	LOD (no. of genomes) ^a	
	Median	Range
NAm 1 (4 isolates)	≤6	1–30
NAm 2 (13 isolates)	≤1	1–6
LAm A (11 isolates)	≤6	1–30
LAm B (5 isolates)	≤6	1–30
Other (6 isolates)	≤6	1–300
Avg	≤6	1–30

^a The number of genomes was calculated by titration of DNA, based on the estimated genome size of *H. capsulatum* of 33 Mb.

H. capsulatum isolates. The LOD was strain dependent, and values fell between 10 fg/μl and 1 pg/μl (1 to 30 genomes per reaction) of *H. capsulatum* (Table 3), with a median of 6 genomes. Sixteen strains were detected at concentrations of ≤100 fg/μl (1 to 6 genomes), and 19 were reactive at ≤10 fg/μl (one genome). When *Hcp100* LAMP was compared with traditional PCR of *Hcp100*, the LAMP assay showed a 10-fold lower LOD (data not shown).

The specificity of the LAMP assay was determined by testing the reactivity of LAMP primers against DNA of other clinically relevant yeasts and molds (Table 2), as well as human and mycobacterial DNA. No cross-reactivity occurred with any other organism tested, and the assay was 100% specific. When the assay incubation time was increased from 1.5 to 2 h, however, cross-reactivity did occur with some negative-control DNA samples.

LAMP assay validity with human urine specimens. The two mock-positive samples that were prepared by spiking control urine samples with known concentrations of *H. capsulatum* DNA were assayed using LAMP and conventional PCR. These samples showed strong signals in the LAMP assay, and *Hcp100* bands were present with PCR amplification (data not shown).

In addition, we tested six urine samples from persons with HIV infection and proven histoplasmosis (Table 4). Four of these samples (67%) showed strong signals in the *Hcp100* LAMP assay, three with fluorescence in both the pellet and supernatant fractions and one with fluorescence in the pellet fraction only (Table 4). None of the 6 samples showed *Hcp100* bands when DNA was amplified using traditional PCR and visualized using ethidium bromide (data not shown). Two samples failed to show amplification with the human β-globin (BG) primers; one of these samples was negative by both the HCP LAMP and BG PCR assays, while one sample was positive by BG PCR but negative by HCP LAMP. Furthermore, one sample that was positive by the LAMP assay failed to show amplification with BG primers. Conversely, no fluorescence was observed in the pellet or supernatant fractions of urine samples from healthy individuals. LAMP amplification products from positive urine samples and isolate DNA were visualized on agarose gels and produced similar characteristic patterns (Fig. 1).

DISCUSSION

The ability to provide laboratory diagnoses in resource-poor regions remains challenging. Many institutions in the developing world do not have the resources to detect and to identify infectious agents rapidly and precisely (20). In the case of PDH associated with HIV, the need for straightforward and inexpensive laboratory diagnostic tools remains important, because PDH can cause death in 95% of cases (21) within months if it is undiagnosed or

TABLE 4 Detection of *H. capsulatum* in human urine specimens using the LAMP assay

Urine specimen no.	Health status ^a	ELISA ^b antigenuria (ng/ μ l)	LAMP result ^c	Human β -globin (PCR)
208	HIV infection, culture-proven histoplasmosis	25.3	+ S, P	+
209	HIV infection, culture-proven histoplasmosis	12.4	—	—
260	HIV infection, culture-proven histoplasmosis	13.8	+ P	+
258	HIV infection, culture-proven histoplasmosis	12.9	—	+
256	HIV infection, culture-proven histoplasmosis	13.1	+ S, P	+
548	HIV infection, culture-proven histoplasmosis	12.6	+ S, P	—
C5	Healthy	0.0	—	+
C6	Healthy	0.0	—	+
C7	Healthy	0.0	—	+
C12	Healthy	0.0	—	+
C13	Healthy	0.0	—	+
C15	Healthy	0.0	—	+
C16	Healthy	0.0	—	+
C17	Healthy	0.0	—	+
UP1	Healthy	0.0	—	+
UP2	Healthy	0.0	—	+

^a Urine specimens were collected from persons with HIV infection and histoplasmosis ($n = 6$) and from healthy control subjects ($n = 10$).

^b ELISA, enzyme-linked immunosorbent assay.

^c S, supernatant fraction; P, pellet fraction.

misdiagnosed. Delayed antifungal treatment of PDH results in mortality rates between 30 and 42% (22–26) in regions where the disease is endemic and where there are underserved populations or limited diagnostic resources. Molecular diagnostic tests have the potential to detect very small amounts of DNA with high specificity, and the LAMP method is both rapid and inexpensive. We developed a LAMP assay to assist in rapid molecular identification of cultured *H. capsulatum* isolates, as well as to detect *H. capsulatum* DNA in urine from patients with disseminated disease. Using DNA from cultured isolates, we showed that the *Hcp100* LAMP assay could detect less than 30 *Histoplasma* genomes, a sensitivity 10-fold greater than that of traditional PCR assays. Further, the LAMP assay did not show cross-reactions with DNA of other fungal organisms or with mycobacteria; the latter cause disseminated infections with symptoms nearly identical to those of PDH in persons with HIV/AIDS.

This novel LAMP assay may have two potential applications for the diagnosis of histoplasmosis in limited-resource settings. First, in a pilot validation study, we found that the LAMP assay was more sensitive than traditional PCR assays in detecting *Hcp100* DNA in urine, and the LAMP assay showed no cross-reactions with DNA isolated from urine specimens from healthy persons, suggesting that this assay can be useful for direct detection of *H. capsulatum* DNA in clinical samples. Second, our data demonstrate that the LAMP assay can be used for rapid confirmation of *H. capsulatum* in culture when the AccuProbe test is not available. The advantages of and caveats for each application are discussed below.

The LAMP method has proven successful in detecting fungal

DNA in specimen types in which whole intact organisms are localized. *Pneumocystis* species DNA has been detected in sputum and bronchiolar lavage (BAL) fluid specimens from patients with pneumonia (27), *Paracoccidioides brasiliensis* DNA has been detected in sputum specimens (28), and *Penicillium marneffei* DNA has been detected in formalin-fixed, paraffin-embedded (FFPE) tissue specimens (29). In our study, we targeted residual intracellular (in leukocyte debris) and free circulating fungal DNA in urine. Obvious advantages of urine are that sample collection is noninvasive and large quantities can easily be obtained from individual patients. Examination of urine sediment reveals a variety of cells including phagocytes (30), in which *H. capsulatum* survives by avoidance of lytic digestion (31). Additionally, DNA released from dying fungal cells is known to cross the renal barrier and is subsequently excreted in urine as cell-free DNA in lengths suitable for detection using PCR (32). In fact, urine is now commonly utilized in molecular diagnostic testing, and many organisms that cause systemic infections are detected in this bodily fluid (33–37).

We tested urine specimens collected from persons with HIV infection who had culture-proven PDH and positive antigenuria to determine LAMP assay sensitivity. Although an obvious caveat of our study is that only a small number of culture-positive urine

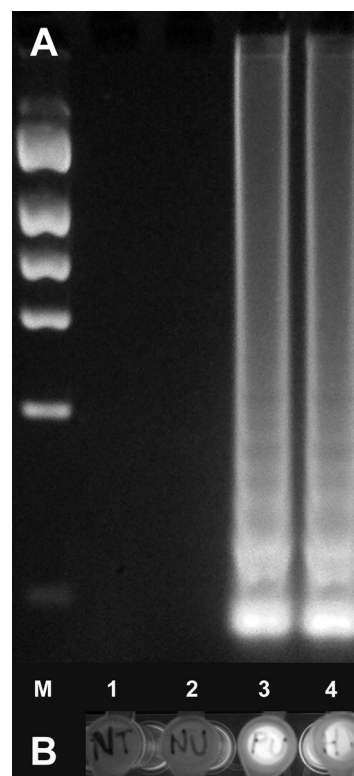


FIG 1 *Hcp100* LAMP assay of DNA from human urine specimens and cultured *H. capsulatum*. (A) LAMP products were visualized on a 1.75% agarose gel. Characteristic “ladder-type” banding is shown with *H. capsulatum* antigen-positive urine and cultured *H. capsulatum* DNA (lanes 3 and 4, respectively), while no DNA amplification was seen after LAMP of healthy control urine (lane 2). Lane M, molecular size marker; lane 1, no-template control. (B) Corresponding tubes were visualized under UV light; fluorescent signals in tubes with antigen-positive urine and cultured *H. capsulatum* DNA are shown (tubes 3 and 4, respectively). Tube 1, no-template control; tube 2, healthy control urine.

specimens were available for testing, these pilot data demonstrated that 67% of samples (4/6 samples) were positive in the *Hcp100* LAMP assay. One of the two urine samples for which negative LAMP results were obtained was also negative with PCR amplification with human β -globin primers, suggesting that no PCR-amplifiable human DNA was present in that sample (Table 4). In our prior experience using FFPE tissue biopsy specimens, we were seldom able to amplify fungal DNA with PCR when the human β -globin locus did not show amplification (38, 39). Human globin DNA could be amplified from a second urine sample that did not react in the LAMP assay. We assume that this sample contained insufficient fungal DNA to be detected even with the sensitive LAMP assay.

We have ruled out the presence of DNA polymerase inhibitors as a cause of insensitivity, since mock-positive urine specimens amplified *Hcp100* strongly in both the LAMP and PCR assays. These samples were spiked with *H. capsulatum* DNA and immediately processed for DNA extraction. All were positive, further suggesting that DNA degradation contributed to decreased sensitivity of LAMP detection in urine. Overall, our data suggest that LAMP can be used to detect *H. capsulatum* DNA in urine samples; however, a large number of urine samples will need to be tested to determine the sensitivity of this method. In addition, the difficulty of extracting high-quality fungal DNA from clinical specimens using a rapid inexpensive method poses the greatest challenge in making LAMP available as a sustainable diagnostic method for fungal infections in resource-challenged laboratories.

The *Hcp100* LAMP assay was highly sensitive in confirming the identification of *H. capsulatum* from DNA prepared from cultured isolates, a feature that can be helpful in countries with limited resources, where culture of blood and/or bone marrow samples is frequently the primary method for diagnosis of PDH. In these countries, diagnostic confirmation of cultured isolates is frequently made using morphological observations alone, and *Histoplasma* can be confused with other yeasts of similar size, such as *Candida glabrata*. Using the *Hcp100* LAMP assay, only a small amount of yeast growth is necessary for DNA extraction and confirmation of *H. capsulatum* in culture.

The purpose of our study was to develop a DNA-based method for detection of disseminated histoplasmosis that could be performed in resource-challenged laboratories. We have shown proof of the concept that LAMP may be a valuable tool for detecting disseminated histoplasmosis. Further evaluation of LAMP using fresh-frozen urine, serum, or whole-blood samples is required, and a simpler and less expensive DNA extraction method should be evaluated for use in resource-limited countries.

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