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Feasibility and Operational Performance of Tuberculosis Detection by Loop-Mediated Isothermal Amplification Platform in Decentralized Settings: Results from a Multicenter Study

Christen M. Gray,[a]* Achilles Katamba,[b] Pratibha Narang,[c] Jorge Giraldo,[d] Carlos Zamudio,[d] Moses Joloba,[e] Rahul Narang,[c] C. N. Paramasivan,[f] Doris Hillemann,[g] Pamela Nabeta,[a] Danielle Amisano,[h]* David Alland,[h] Frank Cobelens,[i,j] Catharina C. Boehme[a]

Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland[a]; Makerere University College of Health Science, Kampala, Uganda[b]; Mahatma Gandhi Institute of Medical Sciences, Sevagram, India[c]; Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru[d]; The National TB Reference Laboratory, Kampala, Uganda[e]; FIND India, New Delhi, India[f]; Forschungszentrum Borstel, National Reference Center for Mycobacteria, Research Center Borstel, Borstel, Germany[g]; Department of Medicine and Center for Emerging Pathogens, Rutgers New Jersey Medical School, Newark, New Jersey, USA[h]; Department of Global Health and Amsterdam Institute for Global Health and Development, Academic Medical Center, Amsterdam, Netherlands[i]; KNCV Tuberculosis Foundation, The Hague, Netherlands[j]

Currently available nucleic acid amplification platforms for tuberculosis (TB) detection are not designed to be simple or inexpensive enough to implement in decentralized settings in countries with a high burden of disease. The loop-mediated isothermal amplification platform (LAMP) may change this. We conducted a study in adults with symptoms suggestive of TB in India, Uganda, and Peru to establish the feasibility of using TB-LAMP (Eiken Chemical Co.) in microscopy laboratories compared with using smear microscopy against a reference standard of solid and liquid cultures. Operational characteristics were evaluated as well. A total of 1,777 participants met the eligibility criteria and were included for analysis. Overall, TB-LAMP sensitivities among culture-positive samples were 97.2% (243/250; 95% confidence interval [CI], 94.3% to 98.2%) and 62.0% (88/142; 95% CI, 53.5% to 70.0%) for smear-positive and smear-negative TB, respectively, but varied widely by country and operator. Specificities ranged from 94.5% (446/472; 95% CI, 92.0% to 96.4%) to 98.0% (350/357; 95% CI, 96.0% to 99.2%) by country. A root cause analysis identified high temperatures, high humidity, and/or low reaction volumes as possible causes for false-positive results, as they may result in nonspecific amplification. The study was repeated in India with training focused on vulnerable steps and an updated protocol; 580 participants were included for analysis. Specificity in the repeat trial was 96.6% (515/533; 95% CI, 94.7% to 97.9%). To achieve acceptable performance of LAMP at the microscopy center level, significant training and infrastructure requirements are necessary.

Approximately 9 million people developed tuberculosis (TB), and 1.5 million people died from the disease in 2013 (1). Substantial progress has been made in increasing treatment success, but 2.9 million of these cases were not diagnosed or diagnosis was not reported due to limited access to diagnostic tests, insufficient sensitivity, or long turnaround time. More rapid and accurate detection at lower levels of care is necessary to reach these lost and missed cases (2). Nucleic acid amplification tests (NAATs) offer speed and sensitivity for pathogen detection, but until recently, no commercial systems had been designed to be simple or inexpensive enough for decentralized settings (3).

Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA), a rapid automated molecular test, has been a major step in the right direction, but initial investment in the equipment and subsequent maintenance remain quite expensive. The development of a less costly approach through the use of manual techniques may expand the application of NAATs.

TB-LAMP is a manual TB detection method that is based on the novel loop-mediated isothermal amplification platform (LAMP) from Eiken Chemical Co. in Japan. LAMP has several features that make it attractive as a diagnostics platform for resource-poor settings. It takes less than 2 h (<60 min of hands-on time), it is less complex than traditional molecular methods, and it generates a fluorescent result that can be detected with the naked eye (4). In a recent study in peripheral laboratories in China using the TB-LAMP assay, the sensitivities in smear-positive, culture-positive sputum specimens and smear-negative, culture-positive sputum specimens were 92.1% (152/165; 95% confidence interval [CI], 86.9% to 95.3%) and 53.8% (113/210; 95% CI, 47.1% to 60.4%), respectively, using solid culture as a reference. The specificity in culture-negative samples was 98.3% (938/954; 95% CI, 97.3% to 99.0%) (5).

In this study, we aimed to evaluate the feasibility and operational characteristics of LAMP in microscopy centers, including

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Address correspondence to Catharina C. Boehme, catharina.boehme@finddx.org.

* Present address: Christen M. Gray, London School of Hygiene and Tropical Medicine, London, United Kingdom; Danielle Amisano, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, New York, USA.

TABLE 1 Description of participating laboratories and primary study population[a]

Characteristic	Location		
	Sevagram, India	Kampala, Uganda	Lima, Peru
Participating laboratories			
No. of laboratories	3	4	4
No. and level of health system (site identification)	3 rural (A, B, C)	2 urban (A, B); 2 rural (C, D)	4 periurban (A, B, C, D)
Distance (range) to reference laboratory (km)	0–60	2–70	20–24
Median LAMP test workload per day (range)	A, B, & C, 6 (1–14)	A & B, 4 (1–12); C & D, 5 (1–14)	A & B, 2 (1–8); C & D, 5 (1–21)
Electricity and backup power	Recurrent power outages (longer at B); generator and UPS	Recurrent power outages. A & D, UPS; B, generator and UPS; C, solar panel	Brief power outages; UPS
Biosafety cabinet	No	Yes (A only)	No
Infrastructure	2 small rooms	A, two medium size rooms, BSC,[b] air conditioner; B, two small rooms (1 container-type); C & D, two medium size rooms	A, one small room with 2 areas; B, one medium size room with 2 areas; C & D, one small room
Laboratory staff	A, 1 lab technician, 1 lab assistant; B, 2 lab technicians; C, 2 lab technicians, 1 lab assistant	A,B,C, & D, 2 lab technicians	A, B, & C, 3 lab technicians; D, 2 lab technicians
Primary study population			
No. of participants included in the analysis	580	719	478
Mean age (range)	42 (18–90)	36 (18–89)	38 (18–84)
Percentage female (no. female/total no.)	32.9 (191/580)	53.5 (385/719)	49.4 (236/478)
HIV prevalence (HIV+ of those with known status) (%)	<3	60.5 (266/440)	<3
Percentage TB prevalence (C+)[c]	17.6 (102/580)	25.6 (184/719)	22.2 (106/478)

[a] Multipurpose labs included microbiology (TB smear microscopy), hematology, biochemistry, and parasitology; working space for TB-LAMP was therefore limited, as bench space often had to be shared. Primary study population characteristics are drawn from study data.
[b] BSC, biosafety cabinet.
[c] C+, culture positive as defined in the Materials and Methods.

infrastructure, training, and environmental concerns, in order to assess their impact on performance in decentralized settings.

MATERIALS AND METHODS

Cohort and setting (primary and repeat studies). In the primary study, we selected 11 microscopy centers with characteristic challenges regarding temperature, human resources, space, and power supply (Table 1). Sites with high TB prevalence rates were deliberately chosen in order to conclude the study in a timely fashion. Only the three microscopy centers in India were included in the repeat trial.

For the primary and repeat studies, adults of ≥18 years of age with symptoms suggestive of pulmonary TB, as defined by national guidelines (typically including a cough of ≥2 weeks and one other TB symptom, such as fever, night sweats, or recent weight loss), were enrolled consecutively. Any individuals with suspected TB who had had TB treatment in the previous 60 days or who were unable to provide at least two sputum samples of ≥1.5 ml were excluded from enrollment.

TB-LAMP operators were local microscopists who had no prior experience with molecular testing. TB-LAMP was set up in two working areas (single or adjacent rooms). The same biosafety precautions were taken as those for smear microscopy.

TB-LAMP procedure. The TB-LAMP procedure consists of the procedure for ultra-rapid extraction (PURE) method (consisting of porous material in a powder-filled tube to adsorb inhibitory agents) and the LAMP reaction tube, containing PCR reagent and primers with high species specification for the *Mycobacterium tuberculosis* complex (details can be found in Fig. 1) (6). Sixty microliters of sputum was transferred to a heating tube using a wide-bore disposable pipette before heat inactivation. The PURE adsorbent tube connects directly to the heating tube and completes the DNA extraction. The primary study specified adding 25 to 35 μl of DNA eluent to the reaction tube for reconstitution, but this was increased to 30 to 35 μl in the repeat trial after a root cause analysis. After 40 min of amplification at 67°C, a visual assessment of the presence or absence of fluorescent light from the reaction tube using UV light determines the test result.

Sample flow and blinding. The two sputa per patient were randomized: one underwent direct smear examination and a TB-LAMP test while the other underwent smear microscopy only. TB-LAMP and smear operators were blinded to the test results of the other. The remainders of the two samples were transported to the reference laboratory on a daily basis for culture testing. Reaction tubes from the TB-LAMP procedure were retained for subsequent analysis. At the reference laboratory, a solid culture (Lowenstein-Jensen [LJ] medium) and a liquid culture (mycobacteria growth indicator tube [MGIT] 960 culture; BD Microbiology Systems) were performed from the same sample as that used for the TB-LAMP after *N*-acetyl-L-cysteine (NALC)-NaOH decontamination (7–9). The first positive culture underwent confirmation for the *M. tuberculosis* complex using MPT64 antigen detection (Capilia TB; Tauns Laboratories) (10).

Root cause analysis. A root cause analysis to examine the parameters potentially associated with TB-LAMP false positives (temperature, humidity, and reaction volume) was carried out. Only the negative control and the TB-LAMP kit were used for this analysis. Using only the negative control, 2 × 200 TB-LAMP tests were carried out with no alterations to the procedure, except that only 20 or 25 μl of eluent was added to the

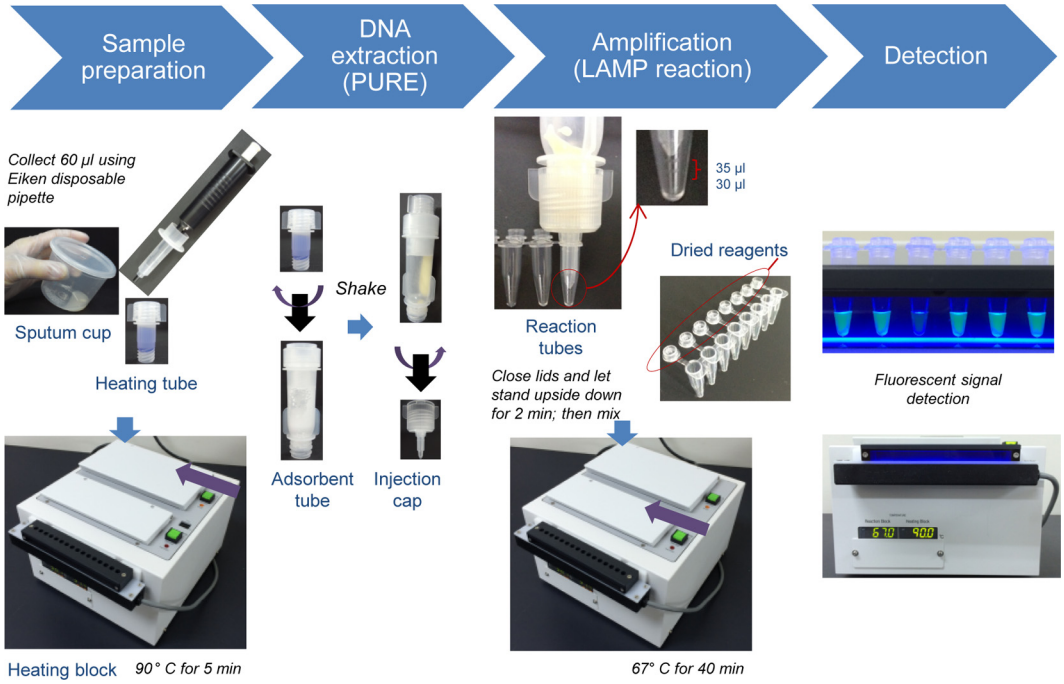

FIG 1 Schematic of the TB-LAMP procedure. A wide-bore disposable pipette is included in the kit to transfer 60 μl of sputum to a heating tube containing extraction solution. A separate room or bench area is used for sputum transfer to minimize the exposure to bacilli and the risk of DNA cross-contamination. The mixture is placed in the heating block at 90°C for 5 min to lyse and inactivate the mycobacteria. After cooling, the heating tube is attached to a tube filled with powder to adsorb inhibitory agents from the lysis mix (PURE method). After mixing, an injection cap is placed on the adsorbent tube to pierce the seal and allow 30 to 35 μl of solution to be dropped into the reaction tube containing the primers.

reaction tubes instead of the specified 25 to 35 μl. Another 6 × 96 tests were similarly performed with the negative control, but these were allowed to sit at 25, 37, or 45°C for 15 min after reconstitution with 25 or 30 μl of eluent. Finally, 8 × 48 tests were performed with the negative control, but the reaction tubes were each exposed to high humidity (85% to 95%) and 25, 37, 40, or 45°C prior to reconstitution with 25 or 30 μl of eluent.

Definitions and diagnostic categorizations. A smear-positive case was defined as at least two smears of scanty grade or one or more smears of 1+ or more as per WHO definitions (11). A culture-positive case was defined as two scanty positive results (a positive MGIT at >28 days from inoculation or an LJ medium with <20 colonies) or any higher positive result that was confirmed to be *M. tuberculosis* complex by identification.

Culture-positive cases identified as *M. tuberculosis* through MPT64 antigen detection were subcategorized into smear-positive and smear-negative samples. Culture-positive cases that were identified as nontuberculous mycobacteria (NTM) were analyzed separately (10). A participant was diagnosed as non-TB if all smear results were negative and all cultures were negative or if one culture was negative and one was contaminated, and the participant was not put on TB treatment based on chest X-ray or clinical discretion. Participants that were smear negative and culture negative and put on TB treatment were considered to have clinical TB and were analyzed separately. If a set of samples collected from the same participant met the following conditions, they were excluded from the analysis: two contaminated or missing cultures; missing *M. tuberculosis* identification or mixed NTM/*M. tuberculosis*; a single scanty positive culture; smear-positive, culture-negative samples; TB-LAMP or smear not done; or missing clinical data.

An indeterminate TB-LAMP reading was defined as the technician being unable to judge whether the test was positive or negative. If a second reader could not judge the result, the test was repeated using the leftover sample.

A batched run referred to a number of samples (2–14) that were run together at one time using a single positive control and a single negative control. Error rates were examined on this basis in order to accurately assess the impact of DNA contamination (determined by a positive negative control) or reagent degradation (determined by a negative positive control).

Data management and analysis. Double data entry was performed locally using a secure, web-based tool developed by the Foundation for Innovative New Diagnostics (FIND). Data analysis was done using SAS 9.2, and 95% confidence intervals were calculated using the Clopper-Pearson binomial exact method (12). Significance testing for variability across operators was also performed using the Clopper-Pearson binomial exact test. Calculations of significant differences in sensitivity or specificity were performed using the Cochran-Mantel-Haenszel test, which was always stratified by country (13).

User training and proficiency testing. Onsite, 5-day standardized TB-LAMP training was carried out by an Eiken-certified trainer in each country just prior to study commencement. Each operator performed three to four TB-LAMP runs before undergoing a proficiency test that was comprised of an observed run with a checklist of key steps to be performed, including cleaning procedures, proper storage and waste procedures, sputum transfer, the PURE method, and the TB-LAMP reaction, followed by a written knowledge test. Failure could stem from low sputum transfer accuracy (determined by weight), a hands-on time of much more than 60 min, failure to perform key steps, or failure of the written exam.

If the operators did not pass initially, additional training, including performing two more runs, was provided followed by another test. In the primary study, proficiency testing was repeated 6 weeks into enrollment to assess the users' abilities to retain skills. In the repeat study, the same format was used, with increased emphasis on the temperature-sensitive steps and the change in reaction volume. In India, two of the three technicians from the primary study remained for the repeat study, but all were given the same new training.

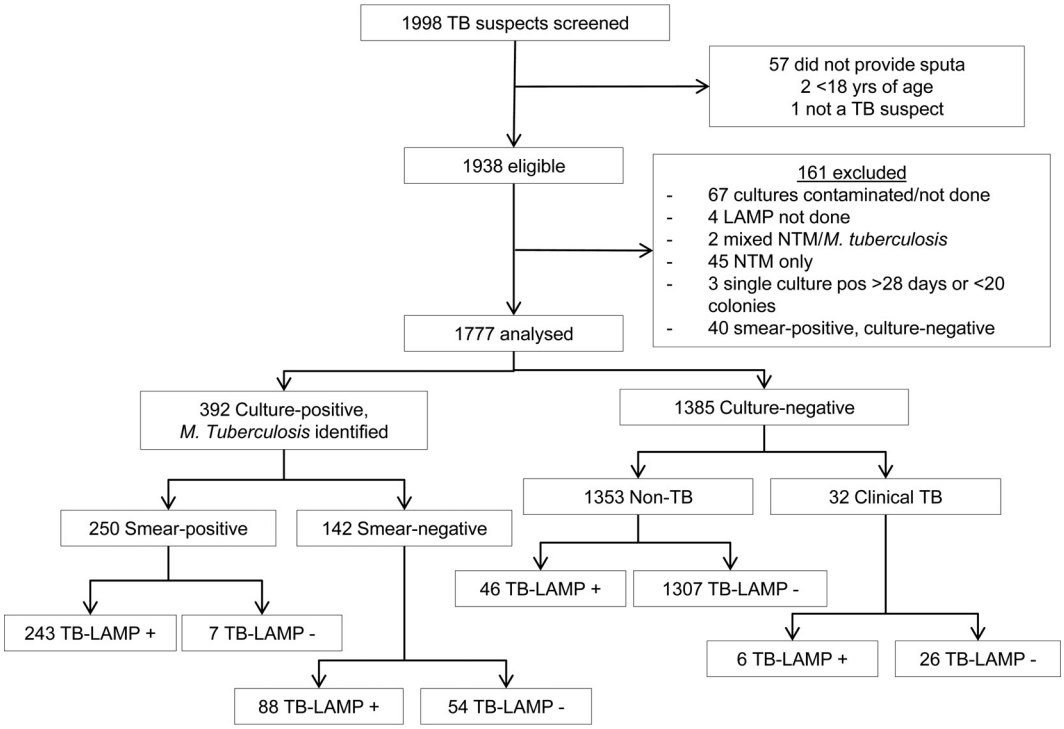

FIG 2 Summary flow chart of patient recruitment and diagnostic testing performed.

Ethical approval. The study was reviewed by the national TB programs of participating countries and was approved by six governing institutional review boards. The requirement to obtain individual informed consent was waived because the standard of care was greater than routine, as culture testing was ordered immediately for all enrollees (not common practice) and results from TB-LAMP were not used to inform treatment.

RESULTS

Results of the primary study. From 8 March 2011 through 11 July 2011, we enrolled 1,938 adults with symptoms suggestive of TB. A total of 161 patients were excluded from the analysis (Fig. 2).

We identified 392 cases of culture-positive TB. The overall sensitivity of TB-LAMP was 84.4% (331/392; 95% CI, 80.5% to 87.9%) (Table 2). In comparison, the sensitivity of routine smear microscopy was 63.8% (250/392; 95% CI, 58.8% to 68.5%). Among those with valid (not missing or contaminated) LJ results, LJ sensitivity was 95.4% (353/370; 95% CI, 92.7% to 97.3%), using all cultures for reference.

TB-LAMP sensitivity in smear-positive, culture-positive samples was 97.2% (243/250; 95% CI, 94.3% to 98.9%); three of the seven false negatives were smear negative for the sputum tested by TB-LAMP. In smear-negative, culture-positive samples, sensitivity was 62.0% (88/142; 95% CI, 53.5% to 70.0%); however, there was significant interoperator variability in detecting smear-negative TB cases (Fig. 3C).

Among HIV-positive patients, the sensitivity of TB-LAMP was 75.0% (45/60; 95% CI, 62.1% to 85.3%) compared with 86.1% (286/332; 95% CI, 82.0% to 89.7%) in HIV-negative patients or individuals with unknown status ($P = 0.59$). Smear microscopy had only 45.0% (27/60; 95% CI, 32.1% to 58.4%) sensitivity in HIV-positive patients and 67.2% (223/332; 95% CI, 61.8% to 72.2%) sensitivity in HIV-negative patients or those with unknown status ($P < 0.01$).

Overall, TB-LAMP specificity in non-TB participants was 96.6% (1,307/1,353; 95% CI, 95.5% to 97.5%). Specificities were 98.0% in Peru (350/357; 95% CI, 96.0% to 99.2%) and 97.5% in Uganda (511/524; 95% CI, 95.8% to 98.7%) but significantly

TABLE 2 TB-LAMP performance against two routine smears, one solid culture and one liquid culture, and species identification where appropriate[a]

Parameter[a]	Point estimate (%) (95% CI), sample size by location			
	India	Uganda	Peru	All sites
Overall sensitivity (C+)	88.2 (80.4–93.8), 102	76.6 (69.8–82.5), 184	94.3 (88.1–97.9), 106	84.4 (80.5–87.9), 392
S+C+	97.3 (90.5–99.7), 73	95.5 (89.7–98.5), 110	100.0 (94.6–100), 67	97.2 (94.3–98.9), 250
S−C+	65.5 (45.7–82.1), 29	48.6 (36.9–60.6), 74	84.6 (69.5–94.1), 39	62.0 (53.5–70.0), 142
HIV− or unknown	88.1 (80.2–93.7), 101	78.0 (69.7–84.8), 127	94.2 (87.9–97.9), 104	86.1 (82.0–89.7), 332
HIV+	100 (2.5–100), 1	73.7 (60.3–84.5), 57	100 (15.8–100), 2	75.0 (62.1–85.3), 60
Overall specificity (S−C−)	94.5 (92–96.4), 472	97.5 (95.8–98.7), 524	98.0 (96–99.2), 357	96.6 (95.5–97.5), 1,353

[a] C+, culture positive; C−, culture negative; S+, smear positive; S−, smear negative; HIV+, HIV positive; HIV−, HIV negative.

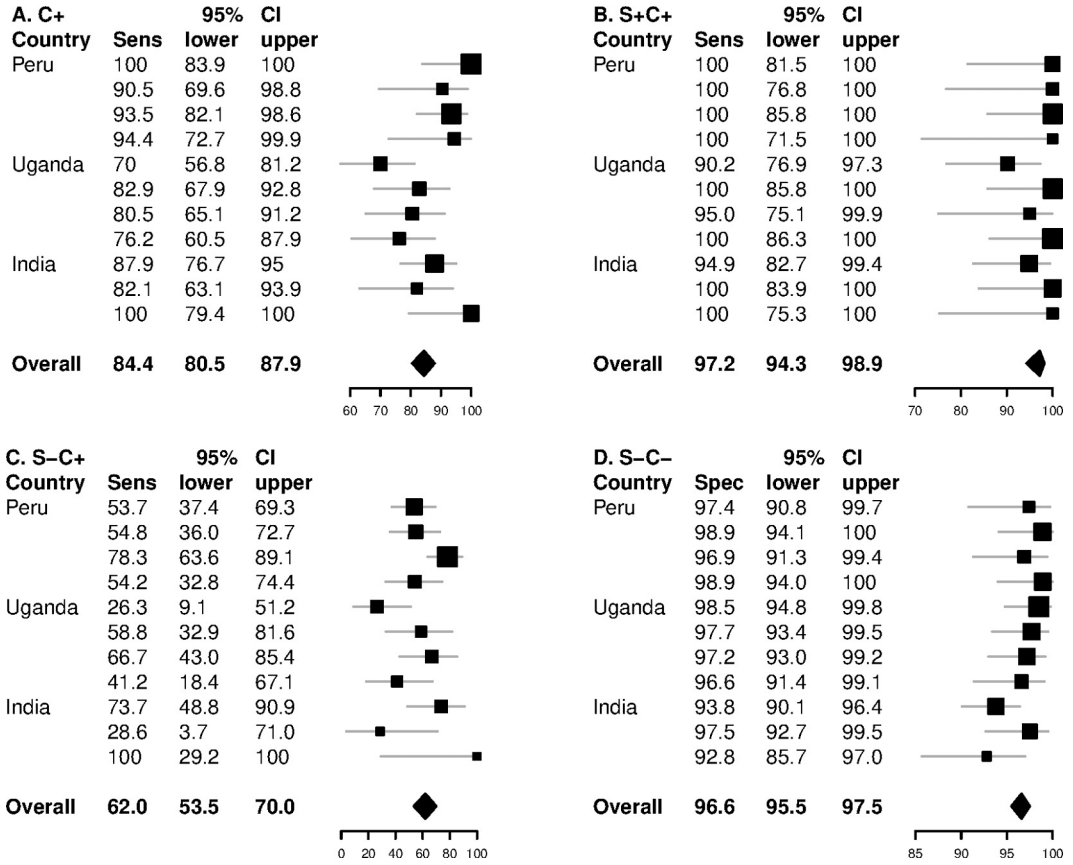

FIG 3 TB-LAMP sensitivity (Sens) and specificity (Spec) by operator for each country. (A) Sensitivity in culture-positive TB cases (C+). (B) Sensitivity in smear-positive, culture-positive cases (S+C+). (C) Sensitivity in smear-negative, culture-positive cases (S−C+). (D) Specificity in non-TB participants (S−C−).

lower in India at only 94.5% (446/472; 95% CI, 92.0% to 96.4%; $P < 0.01$).

Large interoperator variability was observed for the three sites in India ($P < 0.01$) (Fig. 3D). The specificity at site 1 was 93.8% (241/257; 95% CI, 90.1% to 96.4%); at site 2, it was 97.5% (115/118; 95% CI, 92.7% to 99.5%); and at site 3, it was 92.8% (90/97; 95% CI, 85.7% to 97.0%). India also experienced the highest temperatures at the TB-LAMP working stations and for storage conditions (Fig. 4). Specificities in India were 95.7% when ambient temperatures were below 30°C (45/47; 95% CI, 85.5% to 99.5%) and 94.4% when ambient temperatures were above 30°C (401/425; 95% CI, 91.7% to 96.3%; $P = 0.94$). Interoperator variability cannot be explained entirely by temperature, as the operating temperature variation between sites on any given day in India was less than 3°C.

TB-LAMP specificity in those diagnosed with NTM was 92.5% (37/40; 95% CI, 79.6% to 98.4%), excluding clinical TB cases.

The TB-LAMP indeterminate rate across all tests performed was 1.8% (34/1,915). This included DNA contamination events (27 samples affected within 4/533 TB-LAMP batched runs), indeterminate readings (2/1,915 samples), and reagent difficulties signified by a negative positive control (5 samples affected). All indeterminate results were repeated successfully on the same sample. A total of 29/34 indeterminate results occurred in India, and the number of indeterminate results by operator varied sig-

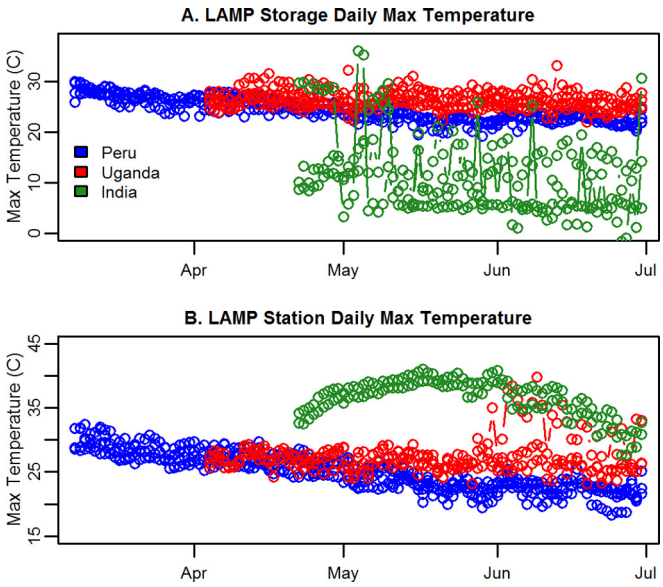

FIG 4 (A) LAMP local daily maximum storage temperature determined by log tag records (LogTag, New Zealand) for each site (3 to 4 per country). Log tags and TB-LAMP reagents were stored in a refrigerator in India. (B) Daily maximum temperature at the TB-LAMP working station where the PURE method and amplification took place.

TABLE 3 Root cause analysis results running the TB-LAMP procedure with only the negative control[a]

Volume DNA eluent (μl)	Temp (°C)	Test results (no. false positive/total no.)	
		Let rxn tubes[b] sit for 15 min after addition of eluent at temp	Let rxn tubes[b] sit for 15 min at temp and high humidity before addition of eluent
25	25	0/96	0/48
	37	1/96	0/48
	40	NP[c]	2/48
	45	1/96	3/48
30	25	0/96	0/48
	37	0/96	0/48
	40	NP	0/48
	45	0/96	0/48

[a] High humidity was 85% to 95% relative to temperature.
[b] rxn tubes, TB-LAMP reaction tubes containing reagents that were reconstituted with the addition of the DNA eluent.
[c] NP, combination was not performed.

nificantly from 0.6% (1/175 runs) to 7.5% (10/133 runs) ($P < 0.01$).

Operational characteristics of LAMP in the primary study. Of the 14 LAMP incubators under 3 years old that were shipped to 11 sites in three countries, only one had to be replaced due to a broken timer, which most likely occurred during shipment. No additional instrument problems were reported.

The number of runs required to pass the proficiency test varied from three to seven (11/14 passed after three runs). By the end of training, all of the operators showed skill and confidence in performing the test. Hands-on time during successful proficiency tests ranged from 30 to 61 min. When proficiency testing was repeated after 6 weeks of enrollment, all passed immediately.

All sites reported brief power outages that lasted from seconds to hours. Uninterruptible power supplies (UPSs) were used in order to be able to complete a whole run once started. In Uganda, longer outages of one to several days were also reported; one site had a solar panel, and the TB-LAMP incubator worked well there during power cuts. However, at another site in Uganda, TB-LAMP testing was halted until a generator was installed.

Plastic waste was treated identically to sputum containers and disposed of according to the local guidelines for hazardous materials.

Manufacturer guidelines stipulate that the storage of reagents and the operation temperature should range from 1 to 30°C. All sites experienced days with operating temperatures above the recommended 30°C, but in India, most days reached over 30°C and many reached over 40°C (Fig. 4). Reagents were stored at the supervisory sites in a fridge or cold room and were distributed to the microscopy laboratories every 2 weeks. In India, after distribution from the supervisory site, short-term storage was also in a refrigerator in order to meet the manufacturer's recommendations.

Root cause analysis. Given the low specificity in India, a root cause analysis was carried out to evaluate the reaction conditions experienced in the field in relation to false-positive rates (FPRs).

Examination of the retained sample reaction tubes suggested that the false positivity may have been related to adding too little of the DNA eluent from the PURE method to the reaction tube (20 to 25 μl). Running TB-LAMP with only the negative control, addition of only 20 to 25 μl eluent into the reaction tubes produced a 1% FPR (4/400 tests).

Given the high temperatures observed at the TB-LAMP stations in India (Fig. 4) and again using only the negative control, after the addition of 25 or 30 μl eluent, the reconstituted reaction reagents were left to set at 25, 37, and 45°C (Table 3). False positives were only observed at ≥37°C and with 25 μl added (4/192, 2.1% FPR).

Reaction tubes were then exposed to various temperatures (25, 37, 40, and 45°C) and high humidity (85% to 95%) for 15 min prior to adding 25 or 30 μl eluent to the reaction tubes (Table 3). False positives only occurred at 40 and 45°C and with 25 μl added (5/96, 5.2% FPR). No other false positives were detected.

Subsequently, a revised TB-LAMP protocol specified a reaction volume of between 30 and 35 μl rather than between 25 and 35 μl and indicated that training should emphasize time and temperature-sensitive steps before and after reconstitution of the TB-LAMP reagents with the DNA eluent. A repeat study was carried out at the same locations in India with the revised protocol to validate these findings.

Results of the repeat study. From 20 January 2012 to 20 March 2012, 699 adults with symptoms suggestive of TB were enrolled, and 580 were included in the analysis. Subjects were excluded as follows: 6 ineligible; 62 inadequate sputum samples; 25 invalid cultures; 3 TB-LAMP not done; 14 NTM or NTM/*M. tuberculosis*; 8 smear-positive, culture-negative; and 1 missing clinical data. TB prevalence in the repeat study was lower (7.2%; 42/580); 35.2% of the participants were female, and the mean age was 40 years (range, 18 to 83 years).

After training with the new protocol, all three operators passed the first proficiency test. TB-LAMP working station temperatures continued to be high (Fig. 5).

No DNA contamination events, indeterminate readings, or reagent problems occurred. TB-LAMP sensitivity in culture-positive patients remained high at 85.7% (36/42; 95% CI, 71.5% to 94.6%). Specificity in non-TB participants rose from 94.5% to 96.6% (515/533; 95% CI, 94.7% to 97.9%; $P = 0.10$, chi-square test). Specificity at site 1 was 96.4% (267/277; 95% CI, 93.5% to 98.3%); at site 2, it was 95.2% (140/147; 95% CI, 90.4% to 98.1%); and at site 3, it was 99.1% (108/109; 95% CI, 95.0% to 100%). Site variability was not statistically significant ($P = 0.84$). Specificity

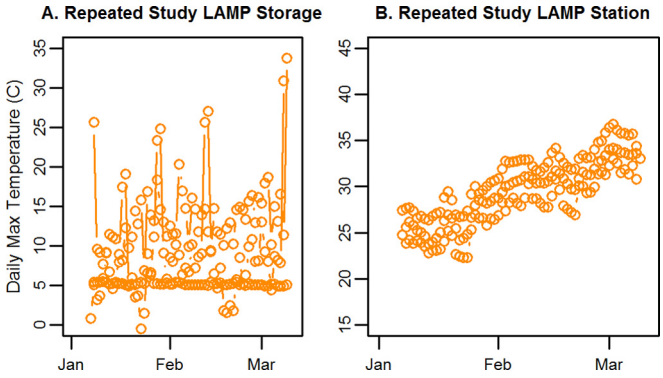

FIG 5 (A) LAMP local daily maximum storage temperature for each of the three sites in India during the repeat study; log tags and TB-LAMP reagents were stored in a refrigerator. (B) Daily maximum temperature at the TB-LAMP working station where the PURE method and amplification took place.

when temperatures were under 30°C was 97.4% (368/378; 95% CI, 95.2% to 98.7%), which is consistent with other countries during the initial study, while specificity when temperatures were over 30°C was still 94.6% (139/147; 95% CI, 89.6% to 97.6%; $P = 0.11$, chi-square test).

DISCUSSION

TB-LAMP showed significant gains in sensitivity over smear microscopy in detecting TB cases in peripheral laboratories, as nearly all smear-positive TB cases (95% to 100%) and roughly 50% to 85% of smear-negative TB cases were detected across the various settings. For comparison, Xpert MTB/RIF, when implemented in urban and periurban health care labs, had sensitivities of 96% to 100% in smear-positive TB cases and 56% to 88% in smear-negative TB cases (14). The interreader variability for TB-LAMP has been reported to be negligible during feasibility studies (6). The indeterminate rate in this study was quite low (1.8%). Users generally found TB-LAMP to be simpler than microscopy and preferable in settings with a standard workload, as it can reach higher throughput through the use of batched runs.

In addition to TB-LAMP, the LAMP method has been used to detect a number of other infectious agents, including malaria, African trypanosomiasis, severe acute respiratory syndrome (SARS), and influenza (15–18). This represents great promise as a cross-disease diagnostic platform.

Because of variability in the concentration of mycobacteria in sputum samples from the same individual, molecular methods, although very sensitive, may not always detect even smear-positive TB cases due to the use of smaller quantities of sputum. TB-LAMP uses only 60 μl of sputum, which may account for the variability in the detection of smear-negative TB cases.

TB-LAMP specificities varied across sites (94% to 98%) and were lower than those for the Xpert MTB/RIF (98% to 100%). Root cause analysis demonstrated a link between false positivity and low reaction volume, especially at high temperatures and time-sensitive steps. A lower reaction volume results in a higher concentration of reagents, which may cause self-priming, particularly at higher temperatures. The volume- and time-sensitive nature of the effect may account for the apparent difference in interoperator performance. Further training on time-sensitive steps and the specification of a higher volume of DNA eluent to reconstitute the TB-LAMP reagents reduced false positives; the repeat study in India after retraining showed an improved specificity of 96.6% overall and a specificity of 97.6% when operating temperatures were under 30°C as recommended by the manufacturer, which is consistent with other sites.

This study was limited in its diagnostic capabilities for non-TB participants, as alternate diagnoses were not recorded and long-term follow-up was not possible. Future long-term implementation studies may be able to incorporate this where programmatically possible for improved specificity estimates.

Infrastructure remains a concern at microscopy centers. Ideally, two separate work areas are required to reduce the risk of DNA contamination. The assay requires only a heat block in terms of equipment, but power supply might be a constraint for this as well as for temperature control.

The intensive training required for TB-LAMP may also be a limitation in laboratories with high staff turnover. Further long-term implementation studies would be required to observe whether this performance can be maintained in routine conditions without retraining.

To our knowledge, this study was the first multicountry assessment of LAMP in peripheral microscopy laboratories and had sufficient power to demonstrate the sensitivity of the manual assay while uncovering problems with specificity. However, the low prevalence of TB cases in the repeat study, due to seasonal variation in clinic attendance, was a possible source of bias. Sample size also limited the power to disambiguate the influence of temperature on specificity from operator error directly through statistical analysis. Further research in settings of intended use would be recommended to further explore and validate these results.

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