INTRODUCTION

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification (NAA) technique that is intended for use in point-of-care settings in developing countries. Since the first report of the LAMP principle in 2004, it has been applied for the detection of various pathogens including *Mycobacterium tuberculosis* (MTB) (1,2). The Loopamp M. tuberculosis complex detection kit (Eiken Chemical Co., Ltd., Tokyo, Japan) was developed specifically for detecting the MTB complex. Its diagnostic performance has been evaluated in several studies both in developed and developing countries (3–6). Currently, the system is widely used as an in vitro diagnostic test in Japan. In 2012, the WHO’s expert group systematically evaluated this method and concluded that further evidence was required to establish its value in clinical practice (3).

The Republic of Haiti is a Caribbean country with a population of 10 million, and its economy is the lowest in the Americas. The incidence rate of TB in Haiti was 206 per 100,000 population, with a case detection rate of smear microscopy and liquid culture in a hospital laboratory in Haiti, which is considered a representative facility for the implementation of this method. The sensitivity, based on culture-positivity, was 86% (95% confidence interval: 81.3–90.3%) and that based on the smear-negative and culture-positive results was 51% (38.7–63.5%). The specificity based on sample negativity for both smear and culture was 98.4% (96.8–99.2). These results are nearly equivalent to those of a clinical study performed in Japan and are comparable with those of other nucleic acid amplification methods. Thus, approximately 18% more tuberculosis patients could be identified by adding the LAMP-TB method to routine smear microscopy in field settings in Haiti. In addition, it is suggested that local technicians could perform LAMP-TB after only short-term training.

SUMMARY: The procedure of ultra-rapid extraction (PURE) and loop-mediated isothermal amplification for tuberculosis (LAMP-TB) is a simple and rapid manual tuberculosis diagnostic with medium-throughput capability. Because of its simplicity, this method could be useful in resource-limited conditions such as microscopy centers in developing countries. This study was conducted to evaluate the clinical performance of this method in a point-of-care setting. The performance was compared to that of smear microscopy and liquid culture in a hospital laboratory in Haiti, which is considered a representative facility for the implementation of this method. The sensitivity, based on culture-positivity, was 86% (95% confidence interval: 81.3–90.3%) and that based on the smear-negative and culture-positive results was 51% (38.7–63.5%). The specificity based on sample negativity for both smear and culture was 98.4% (96.8–99.2). These results are nearly equivalent to those of a clinical study performed in Japan and are comparable with those of other nucleic acid amplification methods. Thus, approximately 18% more tuberculosis patients could be identified by adding the LAMP-TB method to routine smear microscopy in field settings in Haiti. In addition, it is suggested that local technicians could perform LAMP-TB after only short-term training.

MATERIALS AND METHODS

Ethics: Protocols for this study were reviewed and approved by the Ethical Review Board organized by the Haitian Ministry of Public Health and Population, before implementation. Written informed consent was obtained from all participating patients.

Patients and specimens: Patients who visited Grace Children’s Hospital (GCH) in Port-au-Prince, Haiti, and were suspected of having TB were recruited for this study. Patients had to satisfy the following inclusion criteria: persistent cough (> 2 weeks) and at least 1 other common symptom of pulmonary TB (fever, night sweats, malaise, recent weight loss, contact with active case, hemoptysis, chest pain, or loss of appetite), provision of informed consent, and aged 18 years or older.

The exclusion criteria were as follows: patients who had received any anti-TB medication including fluoroquinolones during 60 days before testing, patients with only extra-pulmonary tuberculosis, and patients who...
could not provide informed consent (e.g., the mentally handicapped). Generally, the HIV status was not known by either the patient or the hospital. Between April 26 and June 25, 2013, 227 patients were enrolled in the study.

In accordance with GCH routine clinical evaluation, 2 spot sputum specimens and 1 early morning sputum specimen were collected from each patient within a period of 2 days. For each sample, the participants were requested to submit sputum in a volume of more than 2 mL. The collected sputum specimens were analyzed according to the procedure depicted in the flow chart in Fig. 1.

**LAMP-TB test:** The LAMP-TB test was performed using a sputum processing kit (Loopamp PURE DNA extraction kit; Eiken, Tokyo, Japan) and an MTB amplification detection kit (Loopamp MTBC detection kit; Eiken). The LAMP test was performed according to the manufacturer’s instructions by using 60 μL of neat sputum left over from the smear test, and by smear microscopy technicians at GCH. A dedicated device (a disposable pipette combined with a wide-bore tip, Pipette-60 Set; Eiken) was used for this procedure. The technicians were encouraged to use an appropriate portion of the non-homogenous specimen, as in the case of smear test. The technicians were trained in the operating procedure by one of the authors, an experienced bacteriologist with proficiency in LAMP test procedures as recognized by Eiken. The local technicians had not previously been trained in any molecular techniques. Two days of training was shown to be sufficient to acquire satisfactory technical skill.

**Sputum smear microscopy test:** Fluorescence microscopy was performed by GCH microscopy technicians, using neat sputum specimens according to the routine procedure, and using auramine staining and a light-emitting diode (LED) microscope system. The microscopic findings were reported by grading, from “scanty” to “3” according to WHO guidelines (9).

**Culture examination:** One of the authors performed a culture examination in a biosafety-qualified laboratory at the National Institute of Health in Haiti, which was located approximately 5 km from the GCH. Decontamination was performed on 2 mL of sputum from the remaining specimens that were used for the smear and LAMP tests, using N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH, at concentrations of 0.37% and 2%, respectively) and routine procedures. The collected pellet was resuspended in 500 μL phosphate-buffered saline, and 200 μL was placed in a mycobacteria growth indicator tube (MGIT; Becton-Dickinson Co., Tokyo, Japan) for culture using a Bactec 960 system. Culture specimens that were proven positive within the 6-week incubation period were tested for MTB using an SD TB Ag MPT64 Rapid immunochromatographic identification test (Standard Diagnostics Inc., Seoul, Korea). All of these procedures were performed by a technologist (one of the authors) with decades of experience in TB laboratory work, including molecular technologies, in Japan.

The above 3 types of tests were conducted by independent technicians who were not informed of other test results regarding the specimens that they handled.

**Statistical analysis:** The analyses were performed based on the test results of the smear, culture, and LAMP tests in 1 set (hereafter referred to as “triplet”) for each specimen (per-specimen analysis) irrespective of the source of the specimen. When necessary, analyses were performed based on each individual patient, for example, based on whether or not the subject had any positive test (smear or culture) for TB. Here, a TB patient was defined as a subject for which, a positive smear and/or culture test was obtained using at least 1 specimen. The STARD guidelines (10) were used as a reference in the analyses and the description of the findings.

**RESULTS**

As seen in Fig. 1, 227 patients were enrolled in this
study. Among them, 215 patients submitted 3 sputum specimens following the guidelines; however, 1 patient submitted only 2 specimens, and another 11 patients submitted only 1. Of the 658 specimens submitted, the results of all of triplet examinations were obtained for 472, leaving 186 specimens with at least 1 test result unknown due to culture contamination [117], electrical problem for LAMP [16], or recording error (missing, wrong record) [53]. Complete triplet results for at least 1 specimen were obtained from 209 patients. Of these, 70 had at least 1 positive smear and/or culture for TB, and 139 were negative for both smear and culture. Males accounted for 56% of TB-positive culture specimens; their median age was 39 years with an interquartile range of 36 to 80 years. For 139 cases with both smear and culture negative results, 49 (35%) were males with a median age of 56 years and an interquartile range of 41 to 86 years.

The positive rate, or sensitivity of the LAMP test was 86.5% (134/155) with a 95% CI of 81.3–90.3% in culture-positive specimens. The sensitivity was 99.1% (113/114) for smear-positive and culture-positive specimens, and 51.2% (21/41) for smear-negative and culture-positive-only specimens (for 95% confidence limits, see Table 1).

In total, 317 specimens with triplet examinations were negative by both smear and culture. The LAMP test was negative in 98.4% (312/317) of these specimens, indicating 100% sensitivity and 99.8% specificity (95% CI, 95.3–100%). In contrast, there were 139 subjects with both smear- and culture-negative results, of which, 3 were LAMP positive. Hence, the specificity in bacteriologically TB-negative subjects was 97.8% (136/139, 95% CI, 93.8–99.3%).

Of these negative subjects, 55 had negative test results for 3 consecutive smear and culture examinations. There were no positive LAMP tests in the total 165 specimens (3 × 55 samples), from these cases. This indicates 100% specificity (95% CI, 95.3–100%), using a stricter definition of the gold standard for a negative case.

Although not primarily designed, the time to positive culture (TTP) of MGIT culture test, according to the category of smear and LAMP test results (Figures in parentheses indicate mean TTP ± SD in days, and number of observed subjects).

### Table 1. LAMP-TB test performance in comparison with smear and/or culture examinations, analyses per specimen

| LAMP-TB test | Smear (+) &/or culture (+) | Any | Smear (−) & culture (−) | Likelihood ratio | Predictive value (%) |
|--------------|----------------------------|-----|-------------------------|-----------------|---------------------|
|              | Smear (+) N = 114          | Smear (−) & culture (+) N = 155 | N = 317 |                  |                    |
| Positive     | 113                        | 21                           | 134       |                  |                    |
| Sensitivity (%) | 99.1                        | 51.2                          | 86.5       | 54.8             | 96.4               |
| 95% CI (%)    | 96.2, 99.8                 | 38.7, 63.5                   | 81.3, 90.3 |                  |                    |
| Negative     |                            |                               |            |                  |                    |
| Specificity (%) |                            |                               |            | 98.4             | 0.14               |
| 95% CI (%)    |                            |                               |            | 96.8, 99.2       |                    |

Note: There was no specimen with smear (+) and culture (−).

CI, Confidence interval. Likelihood ratio and predictive values are calculated based on positive or negative rates according to “any” positives (smear-positive or culture positive).
The limit of detection of the LAMP test, in terms of TTP for MGIT culture, lay between the boxes for S−/L+ and S−/L−, and was approximately 15 days. Regarding the technical requirement for handling the LAMP test, after 2 days training, all local technicians engaged in the work reported no difficulty in doing this work throughout the entire testing period. Their work was periodically supervised by one of the authors, and no faults were detected, other than several cases of unavoidable failure due to trouble with the electric power supply.

**DISCUSSION**

The WHO’s expert committee previously reported the results of 3 series of studies by FIND on the performance of the LAMP test (3). The sensitivity was 96.9% for smear-positive and culture-positive specimens, 71.4% for smear-negative and culture-positive specimens, and 89.1% for all culture-positive specimens in the “FIND 2nd Evaluation studies” in India (2012). In the “FIND Evaluation studies (intended setting of use)” in India, Uganda, and Peru (2011), these values were 97.2%, 62.0%, and 84.4%, respectively. Another series, the “FIND Evaluation studies (reference laboratories)” in South Africa, Peru, and Brazil (2010) reported values of 97.2%, 53.1%, and 77.7%, respectively. Later Mitarai et al. reported similar findings from Japan, specifically, 98.2%, 55.6%, and 87.9%, respectively (for direct, non-decontaminated/digested specimens, in accordance with the FIND series above) (4). Another prototypic LAMP test trial was performed with point of care (POC) conditions using an operational study design (5). The results, based on the aforementioned metrics, showed sensitivity values of 97.7%, 48.8%, and 88.2% respectively.

Our findings corresponding to these sensitivity parameters were 99.1%, 51.2%, and 86.5%, respectively, which could be considered roughly similar to those of the preceding studies, which was despite wide difference in operational conditions among the studies. This indicates that the LAMP-TB test could be practiced in resource-limited settings.

Variability was seen in the positive rates for smear-negative and culture-positive specimens. One possible reason for the relatively low rate identified in our study is the use of LED-fluorescence microscopy, which is more sensitive than conventional microscopy (11). The use of this or another type of microscopy was not clearly stated in other similar reports.

The specificity was computed based on negative specimens for both the smear and culture assays. The WHO reported the specificity of the study by Mitarai et al. as 90.7%, simply based on the LAMP-negative rate among culture-negative specimens (3), whereas Mitarai reported specificity of 99.9%, based on the number of patients diagnosed bacteriologically as well as clinically (4). We had no false-positive cases after applying a strict definition of a non-TB person, namely 3 consecutive negative tests. Attention should be paid to the definition and strictness of a “negative” specimen/person classification for calculating specificity.

In addition, Mitarai et al. (4) suggest that the source of false-positives might be cross-contamination in the laboratory (12) or at the bedside, which should also be considered, especially when contemplating the otherwise very low frequency of false positives inherent to the LAMP test.

Overall, the use of the LAMP-TB test could increase the detection of TB by 18% (21/114), in comparison to LED microscopy. This is similar to our preliminary results in Haiti (6), wherein we assessed the use of this method together with smear, but without culture examination, and found that of 440 patients tested (by both tests), 86 were smear-positive, and 16 were smear-negative and LAMP-positive; therefore, the marginal gain of LAMP was 19% (16/86).

Thus, this study confirmed that the performance of the LAMP test was similar to that of previous reports from studies using different conditions, such as an advanced country (4) or a multi-country trial setting (3). This can be compared to our study obtained specimens from a single country, in a near-routine and technically primitive setting.

GeneXpert (Cepheid, Sunnyvale, CA, USA) is another system of POC NAA that has been endorsed by the WHO and is widely used (13). Recently Walusimbi et al. (14) reviewed its diagnostic performance and reported that its pooled sensitivity for smear-negative, culture-positive cases, based on 15 different reports, was 67%, and that the specificity was 98%, which are again comparable to the LAMP-TB test results of this study.

The relationship between the time to positive culture of bacillary growth and the original bacterial numbers, in terms of colony-forming units (CFU) per volume, was established by Bark et al. (15), with a TTP of 15 days corresponding roughly to 100 CFU/mL; this is also the lower limit of detection of LAMP, as seen in Fig. 2. This is similar to the observed detection limit of 131 CFU/mL for GeneXpert (16), supporting the similarity in detection capabilities between LAMP-TB and GeneXpert, apart from the latter test’s additional important function of detecting rifampicin resistance.

There are some limitations to this study. There were a considerable number of specimens excluded from analyses due to contamination and errors in recording. Although these failures seemed to have occurred randomly, any possible bias cannot be excluded that could have affected the results of the evaluation. Second, the study did not analyze the possible influence of HIV infection, which is as common as 20% among TB patients in this country. The utility of the LAMP method for detecting the paucibacillary type of TB in such populations would be a strong rationale for this method, and therefore this issue remains a highly relevant challenge. In addition, the TTP analysis of MGIT has some weaknesses including possible disagreement between the bacillary load in an individual and that of his/her sputum specimen.

In conclusion, the LAMP-TB test, a new POC diagnostic that exhibits performance comparable to that of other NAA tests, and does not require sophisticated skill, could be used to respond to the needs for better TB diagnostics, as outlined in the Post-2015 Global TB Strategy (17).
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Conflict of interest None to declare.

REFERENCES

1. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28: E63.
2. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother. 2009;15:62-9.
3. WHO Expert Group Meeting Report Geneva: May 2013. The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis. WHO/HTM/TB/2013.5
4. Mitarai S, Okumura M, Toyota E, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2011;15:1211-7.
5. Boehme CC, Nabeta P, Henostroza G, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol. 2007;45:1936-40.
6. Kaku T, Shirasu N, Yoshida M, et al. Feasibility of TB-LAMP, a simple and rapid nucleic acid amplification test (NAAT), in Haiti. 44th World Conference on Lung Health of the International Union Against Tuberculosis and Lung Disease (The Union); 2013 October 30–November 3; Paris, France.
7. WHO. Global tuberculosis report 2014. World Health Organization, Geneva, 2014. (supplemented with; Tuberculosis Country Profile. Available at <http://www.who.int/tb/data>. Accessed September 2015.
8. Cattamanchi A, Dowdy DW, Davis JL, et al. Sensitivity of direct versus concentrated sputum smear microscopy in HIV-infected patients suspected of having pulmonary tuberculosis. BMC Infect Dis. 2009;9:53.
9. WHO. Laboratory Services in Tuberculosis Control. Part II: Microscopy. Geneva: WHO; 1998.
10. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med. 2003;138:W1-W12.
11. WHO. Fluorescent Light-emitting Diode (LED) Microscopy for Diagnosis of Tuberculosis: Policy Statement. Geneva: WHO; 2011.
12. van Deun JM, Pijnenburg JE, van Rijswoud CM, et al. Investigation of cross contamination in a Mycobacterium tuberculosis laboratory using IS6110 DNA fingerprinting. Int J Tuberc Lung Dis. 1998;2:425-9.
13. WHO. Policy Statement: Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampin Resistance: Xpert MTB/RIF System. 2011. Available at <http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf?ua=1>. Accessed July, 2014.
14. Walusimbi S, Bwanga F, De Costa A, et al. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infect Dis. 2012;13:507.
15. Bark CM, Okwera A, Johola ML, et al. Time to detection of Mycobacterium tuberculosis as an alternative to quantitative cultures. Tuberculosis (Edinb). 2011;91:257-9.
16. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48:229-37.
17. WHO. Draft Global Strategy and Targets for Tuberculosis Prevention, Care and Control after 2015: Report by the Secretariat. 2014. Available at <http://apps.who.int/gb/ebwha/pdf_files/WHA67/A67_11-en.pdf>. Accessed July 21, 2014.