Diagnostic Accuracy of the PURE-LAMP Test for Pulmonary Tuberculosis at the County-Level Laboratory in China

Xichao Ou1,*, Qiang Li1,*, Hui Xia1,*, Yu Pang1, Shengfen Wang1, Bing Zhao1, Yuanyuan Song1, Yang Zhou1, Yang Zheng1, Zhijian Zhang5, Zhiying Zhang2, Junchen Li2, Haiyan Dong2, Jack Zhang2, Kai Man Kam3, Junying Chi4, Shitong Huan4, Daniel P. Chin4,*, Yanlin Zhao1,*

1 National Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, P. R. China, 2 PATH, China Office, Beijing, China, 3 Stanley Ho Centre for Emerging Infectious Diseases, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, P. R. China, 4 Bill & Melinda Gates Foundation, China Office, Beijing, China, 5 Respiratory Diseases Department of Nanlou, Chinese People’s Liberation Army General Hospital, Beijing, P. R. China

Abstract

Background: Early and effective detection of Mycobacterium tuberculosis (MTB), particularly in smear-negative tuberculosis (TB), is a priority for global TB control. Loop-mediated isothermal amplification with a procedure for ultra rapid DNA extraction (PURE-LAMP) can detect TB in sputum samples rapidly and with high sensitivity and specificity. However, the PURE-LAMP test has not been effectively evaluated, especially in resource-limited laboratories. In this study, we evaluated the performance of the PURE-LAMP test for TB detection in TB suspects from two county-level TB dispensaries in China.

Methodology/Principal Findings: From April 2011 to February 2012, patients with suspected TB were continuously enrolled from two county-level TB laboratories in China. Three sputum samples (spot, night, and morning sputum) were collected from each recruited patient. Detection of MTB by PURE-LAMP was compared to a reference standard L-J culture. The results showed that the sensitivity of the PURE-LAMP test based on spot sputum for MTB detection was 70.67%, while the sensitivity of the PURE-LAMP test based on spot sputum for MTB detection in smear positive and culture positive patients was 92.12% and 53.81%, respectively. The specificity of PURE-LAMP based on spot sputum for MTB detection was 98.32%. The sensitivity and specificity of the PURE-LAMP test based on three sputa combination for MTB detection was 88.80% and 96.86%, respectively. The results also showed that the PURE-LAMP test had a significantly lower contamination rate than did solid culture.

Conclusions/Significance: The study suggested that, in peripheral-level TB laboratories in China, the PURE-LAMP test showed high sensitivity and specificity for TB detection in TB suspects, making it a more effective, rapid, and safe method worthy of broader use in the future.

Introduction

Tuberculosis (TB) remains a major global health problem. In 2012, an estimated 8.6 million people developed TB and 1.5 million died from the disease [1]. Accurate and rapid diagnosis of TB is vitally important in establishing appropriate clinical management and infection control measures [2,3]. Currently, the most common method for TB diagnosis worldwide is sputum smear microscopy, the sensitivity of which is notoriously poor, particularly in human immunodeficiency virus (HIV)-positive patients [4,5]. Culture, the gold standard diagnostic method, is highly sensitive but takes between two and six weeks to obtain a result [2]. To address the need for rapid and sensitive diagnosis of TB, a number of nucleic acid amplification assays have been invented [6,7,8]; however, they are still not routinely applied in developing countries due to their high cost, complicated procedures, insufficient laboratory facilities, and a shortage of skilled technologists [9,10,11].

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that does not require an expensive thermocycler or detection system [12]. TB-LAMP is a new manual TB detection method based on the LAMP platform from Eiken Chemical Company in Japan. TB-LAMP has several features that make it attractive as a diagnostics platform for resource-poor settings: it is fast (40 minutes), isothermal (requiring only a heat block), robust to inhibitors and reaction conditions that usually adversely affect polymerase chain reaction methods, and it generates a result that can be detected with the naked eye.
From April 2011 to February 2012, 1378 eligible TB suspects were enrolled. No patients were excluded from the study because of insufficient specimen to conduct all the 3 tests. For analysis, 46 patients were excluded because of culture contamination, and 3 patients were excluded because of no sequence result, therefore, only 1329 TB suspects were remained for analysis (Figure 1). Of these 1329 TB suspects, 888 were male, 441 were female; 53 of the patients were aged 20 years and under; 165 (12.42%) were smear positive and culture positive TB patients, 210 (15.80%) were smear-negative and culture-positive TB patients, and 954 (71.78%) were culture negative TB patients (Table 1).

Performance of PURE-LAMP Test for MTB Detection Based on Spot Sputum

Among the 1329 analyzed TB suspects, the sensitivity and specificity of PURE-LAMP for MTB detection based on spot sputum were analyzed using solid culture as the reference standard (Table 2, Figure 1). The diagnostic results of 1203 TB suspects (90.52%) were consistent with those by solid culture. The sensitivity of PURE-LAMP based on spot sputum was 70.67%, while the sensitivity of PURE-LAMP based on spot sputum in smear-positive and culture positive TB patients was 92.12%. The specificity of PURE-LAMP for MTB detection based on spot sputum in smear-negative and culture positive patients was 53.01%. The specificity of PURE-LAMP for MTB detection based on spot sputum was 98.32%.

Performance of PURE-LAMP for MTB Detection Based on Different Sputum and Sputum

Of the 1329 TB suspects analyzed, we calculated the sensitivity and specificity of PURE-LAMP based on different sputum (spot sputum, night sputum or morning sputum) or different sputum combination (spot and night sputum, spot and morning sputum, night and morning sputum, spot and night and morning sputum) (Table 3). The sensitivity and specificity of PURE-LAMP based on the number and combinations of sputum specimens collected were
calculated using solid culture as a reference standard (Table 3). The sensitivity of PURE-LAMP for MTB detection based on three sputa is higher than that based on one sputum specimen.

Contamination Rate of PURE-LAMP and Culture

In this study, 475 runs of the PURE-LAMP were conducted on new patients with suspected pulmonary TB. Among the 475 runs of PURE-LAMP, only 1 run was contaminated, with a total contamination rate of 0.21%.

A total of 4,129 sputum specimens were collected from the 1,378 patients enrolled at the two dispensaries, with each digested specimen inoculated into two tubes of culture medium. Among the 8,268 cultures, 276 tubes were contaminated, with a total contamination rate of 3.3%.

Analysis of Discrepant Cases

We analyzed the discrepant cases and found that among the 30 culture-negative patients who were diagnosed positive by PURE-LAMP, 2 patients were smear-positive. Of the 42 culture-positive patients who were diagnosed as negative by PURE-LAMP based on three samples, 6 were identified as NTM by sequence, while 36 were identified as MTB by sequence. All the 36 MTB strains were smear-negative and twenty-six of these patients (72.22%) had a colony number of less than 20 for solid culture.

Discussion

Early diagnosis is important for the control and prevention of TB. With the development of molecular biology, a number of methods have been developed for the rapid detection and diagnosis of TB. LAMP is a new nucleic acid amplification method that allows for rapid and sensitive detection of TB. This study evaluated the diagnostic accuracy of PURE-LAMP and compared it with solid culture as the gold standard.

Table 1. Demographic information for the participants who were analyzed.

| Variable                  | Xinxiang (N = 902) | Huojia (N = 427) | All patients (N = 1329) |
|---------------------------|--------------------|-----------------|------------------------|
| Age range                 |                    |                 |                        |
| <20                       | 31 (3.44)          | 22 (5.15)       | 53 (3.99)              |
| 20–39                     | 369 (40.91)        | 121 (28.34)     | 490 (36.87)            |
| 40–59                     | 295 (32.71)        | 153 (35.83)     | 448 (33.71)            |
| ≥60                       | 207 (22.95)        | 131 (30.68)     | 338 (25.43)            |
| Gender                    |                    |                 |                        |
| Female                    | 280 (31.04)        | 161 (37.70)     | 441 (33.18)            |
| Male                      | 622 (68.96)        | 266 (62.30)     | 888 (66.82)            |
| Culture result            |                    |                 |                        |
| Smear-positive/culture-positive | 117 (12.97)   | 48 (11.24)      | 165 (12.42)            |
| Smear-negative/culture-positive | 186 (20.62)     | 24 (5.62)       | 210 (15.80)            |
| Culture-negative          | 599 (66.41)        | 355 (83.14)     | 954 (71.78)            |

The sensitivity of PURE-LAMP for MTB detection based on three sputa is higher than that based on one sputum specimen.
technology first proposed by Notomi in 2000 [12]. The PURE-LAMP test is a new, simple, contamination-resistant kit for the diagnosis of TB.

In this study, the sensitivity of PURE-LAMP based on spot sputum in smear-negative and culture-positive patients was 53.81%, the overall sensitivity of PURE-LAMP was 70.67%, and the specificity of PURE-LAMP was 98.32%, which was similar with that found in other studies [11]. Comparing the sensitivity of PURE-LAMP with that of smear microscopy, the sensitivity of PURE-LAMP was higher than the sensitivity of smear microscopy. This result showed that PURE-LAMP can be used for MTB diagnosis.

Chinese national guidelines have recommended obtaining three sputum specimens from patients with suspected TB [21]. In the present study, we assessed the contribution of each specimen collected to the ultimate diagnosis of MTB for TB suspects and found that the sensitivity of the PURE-LAMP test for MTB detection in TB suspects from three sputa was significantly higher than that in one test of spot sputum. Considering the cost factor, testing three samples may increase the economic burden of

### Table 2. Performance of the PURE-LAMP test for TB detection in TB suspects based on spot sputum.

| Site and no. of test | Site and no. of test | Site and no. of test | Site and no. of test | Site and no. of test | Site and no. of test |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| All culture positive | Smear positive and culture positive | Smear negative and culture positive | Specificity (no tuberculosis) | PPV | NPV |
| Xinxiang Correct-no./total no. (%)205/303(67.66) | 104/117(88.89) | 101/186(54.30) | 587/599(98.00) | 205/217(94.47) | 587/685(85.69) |
| Huojia Correct-no./total no. %60/72 (83.33) | 48/48(100) | 12/24 (50.00) | 351/355(98.87) | 60/64(93.75) | 351/363(96.70) |
| All patients Correct-no./total no. (%)265/375(70.67) | 152/165(92.12) | 113/210(53.81) | 938/954(98.32) | 265/281(94.31) | 938/1048(89.50) |

### Table 3. Performance of PURE-LAMP based on different sputum and different sputum combination.

| No. of TB test | Sensitivity | Specificity (No-tuberculosis) | PPV | NPV | Diagnostic accuracy |
|---------------|-------------|--------------------------------|-----|-----|---------------------|
| 3 samples (spot, morning, and night sputum) | | | | | |
| Correct-no./total no. (%)333/375(88.80) | 924/954(96.86) | 333/363(91.74) | 924/966(95.65) | 1257/1329(94.58) |
| 95% CI | (85.21–91.61) | (95.55–97.79) | (88.45–94.15) | (94.18–96.77) | (93.23–95.68) |
| 2 samples | | | | | |
| Spot and night sputum | Correct-no./total no. (%)310/375(82.67) | 929/954(97.38) | 310/335(92.54) | 929/994(93.36) | 1237/1329(93.23) |
| 95% CI | (78.51–86.16) | (96.16–98.22) | (89.22–94.89) | (91.75–94.84) | (91.75–94.46) |
| Spot and morning sputum | Correct-no./total no. (%)309/375(82.40) | 928/954(97.27) | 309/335(92.24) | 928/994(93.36) | 1237/1329(93.08) |
| 95% CI | (78.22–85.92) | (96.04–98.13) | (88.87–94.65) | (91.64–94.75) | (91.58–94.32) |
| Night and morning sputum | Correct-no./total no. (%)314/375(83.73) | 931/954(97.59) | 314/337(93.18) | 931/992(93.85) | 1245/1329(93.68) |
| 95% CI | (79.66–87.12) | (96.41–98.39) | (89.97–95.41) | (92.18–95.18) | (92.24–94.87) |
| 1 sample | | | | | |
| Spot sputum | Correct-no./total no. (%)265/375(70.67) | 938/954(98.32) | 265/281(94.31) | 938/1048(89.50) | 1203/1329(90.52) |
| 95% CI | (65.87–75.05) | (97.29–98.97) | (90.95–96.47) | (87.50–91.22) | (88.83–91.98) |
| Night sputum | Correct-no./total no. (%)267/375(71.20) | 938/954(98.32) | 267/283(94.35) | 938/1046(89.67) | 1205/1329(90.67) |
| 95% CI | (66.42–75.53) | (97.29–98.97) | (91.01–96.49) | (87.68–91.38) | (88.99–92.12) |
| Morning sputum | Correct-no./total no. (%)261/375(69.60) | 935/954(98.01) | 261/280(93.21) | 935/1049(89.13) | 1196/1329(89.99) |
| 95% CI | (64.76–74.04) | (96.91–98.72) | (89.65–95.61) | (87.10–90.88) | (88.26–91.49) |
patients. Therefore, it is necessary to conduct an analysis of the cost-effectiveness of the PURE-LAMP test with different specimen combinations in the future.

The contamination rate of PURE-LAMP was 0.21% in this study. Two labs in this study were both equipped with three independent and separate working areas, including the storage area for reagents, the pretreatment area, and the amplification and results analysis area. Each room was clearly labeled to avoid confusion when retrieving equipment or materials from different areas. Work benches were disinfected by a 5% sodium hypochlorite solution and exposed to UV light after each testing. Through strict control of contamination and the closed system of the PURE-LAMP test, the risk of contamination was highly reduced.

We also found PURE-LAMP’s reporting time much shorter than that of solid culture: a diagnosis can be completed within two hours. Fluorescence results were examined with the naked eye by lab technicians, and no indeterminate cases were found. China’s NTRL rechecked 20% of the sample results, and the concordance rate was 100%.

In conclusion, the PURE-LAMP test is a new diagnostic technology that can rapidly and accurately detect TB in patients.

References

1. World Health Organization (2013) Global tuberculosis control: WHO report 2011. Geneva, Switzerland: World Health Organization.
2. Small P M, Pai M (2010) Tuberculosis diagnosis-time for a game change. N Engl J Med 363: 1070–1071.
3. Pai M, Kalantri S, Dhelda K (2006). New tools and emerging technologies for the diagnosis of tuberculosis: part II. Active tuberculosis and drug resistance. Expert Rev Mol Diagn 6: 423–432.
4. Elliott AM, Halwiindi B, Hayes RJ, Luo N, Tembo G, et al. (1993) The impact of human immunodeficiency virus on presentation and diagnosis of tuberculosis in a cohort study in Zambia. J Trop Med Hyg 96: 1–11.
5. Klein NC, Duncanson FP, Lenox M, Story E, Boehme C, Wallace E, et al. (2010) Rapid detection of mycobacterial smears in the diagnosis of pulmonary tuberculosis in AIDS/ARC patients. Chest 95: 1190–1192.
6. Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, et al. (1993) Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J Clin Microbiol 31: 3270–3274.
7. Helb D, Jones M, Storty E, Boehme C, Wallace E, et al. (2010) Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48: 229–237.
8. Guo Y, Zhou Y, Wang C, Zhu L, Wang S, et al. (2009) Rapid, accurate determination of multidrug resistance in M. tuberculosis isolates and sputum using a biochip system. Int J Tuberc Lung Dis 13: 914–920.
9. Huggett JF, McHugh TD, Zumla A (2003) Tuberculosis: amplification-based clinical diagnostic techniques. Int J Biochem Cell Biol 35: 1407–1412.
10. Sarmiento OL, Weigle KA, Alexander J, Weber DJ, Miller WC (2003) Assessment of novel in-house loop-mediated isothermal amplification (LAMP) assay for detection of Mycobacterium tuberculosis in sputum samples from patients with pulmonary tuberculosis. J Clin Microbiol 41: 3233–3240.
11. Suffi P, Palomino JC, Gardaso Lezo S, Equtia C, Cataldi A, et al. (2000) Evaluation of the polymerase chain reaction for the detection of Mycobacterium tuberculosis. Int J Tuberc Lung Dis 4: 179–183.
12. Notomi T, Okazama H, Masubuchi H, Yonekawa T, Watanabe K, et al. (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28: 63–65.
13. Adhikari BR, Pandey BD, Ghimire P, Shrestha B, Khadka M, et al. (2009) Loop-mediated isothermal amplification (LAMP) for the direct detection of human pulmonary infections with environmental (nontuberculosis) mycobacteria. Jpn J Infect Dis 62: 212–214.
14. Pandey BD, Poudel A, Yoda T, Tamara A, Oda N, et al. (2008) Development of an in-house loop-mediated isothermal amplification (LAMP) assay for detection of Mycobacterium tuberculosis and other nontuberculosis mycobacteria. J Clin Microbiol 46: 407–412.
15. Ikewada T, Sonobe T, Honda K (2003) Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis in clinical specimens. Methods Mol Med 6: 423–432.
16. Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, et al. (1993) Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J Clin Microbiol 31: 3270–3274.
17. Helb D, Jones M, Storty E, Boehme C, Wallace E, et al. (2010) Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48: 229–237.
18. Guo Y, Zhou Y, Wang C, Zhu L, Wang S, et al. (2009) Rapid, accurate determination of multidrug resistance in M. tuberculosis isolates and sputum using a biochip system. Int J Tuberc Lung Dis 13: 914–920.
19. Huggett JF, McHugh TD, Zumla A (2003) Tuberculosis: amplification-based clinical diagnostic techniques. Int J Biochem Cell Biol 35: 1407–1412.
20. Sarmiento OL, Weigle KA, Alexander J, Weber DJ, Miller WC (2003) Assessment of novel in-house loop-mediated isothermal amplification (LAMP) assay for detection of Mycobacterium tuberculosis in sputum samples from patients with pulmonary tuberculosis. J Clin Microbiol 41: 3233–3240.
21. Suffi P, Palomino JC, Gardaso Lezo S, Equtia C, Cataldi A, et al. (2000) Evaluation of the polymerase chain reaction for the detection of Mycobacterium tuberculosis. Int J Tuberc Lung Dis 4: 179–183.

The test used only 60 μL of sputum each time and was very suitable for pulmonary TB patients, especially for those patients who lacked sputum. Our field study proved that the PURE-LAMP test could be used for screening TB patients in labs in China’s periphery in the future.

Acknowledgments

We gratefully thank the Bill & Melinda Gates Foundation for its financial support, and PATH for its assistance in implementing the project, analyzing data, and writing this paper. We also thank all staff in project laboratories that contributed to this work.

Author Contributions

Conceived and designed the experiments: XO QL HX Zhiving Zhang Y. Zhao. Performed the experiments: XO YP SW BZ YS Y. Zhou Y. Zheng Zhijian Zhang. Analyzed the data: XO HD JZ KMK JC Y. Zhao. Contributed reagents/materials/analysis tools: JL SH DPC. Wrote the paper: XO QL HX Y. Zhao.