Tuberculosis 94 (2014) 428–433

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

DIAGNOSTICS

A multicenter study of Cross-Priming Amplification for tuberculosis diagnosis at peripheral level in China

Xichao Ou [a,1], Yuanyuan Song [a,1], Bing Zhao [a,1], Qiang Li [a], Hui Xia [a], Yang Zhou [a], Yu Pang [a], Shengfen Wang [a], Zhijian Zhang [b], Shiming Cheng [a], Changting Liu [b,**], YanLin Zhao [a,*]

[a] Chinese Center for Disease Control and Prevention, Beijing, China
[b] Chinese PLA General Hospital, Beijing, China

ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form
18 April 2014
Accepted 26 April 2014

Keywords:
Mycobacterium tuberculosis
CPA
Sensitivity
Specificity

SUMMARY

Cross-Priming Amplification (CPA) has been shown to rapidly and effectively detect *Mycobacterium tuberculosis* (MTB) in sputum samples under isothermal conditions. However, no performance data exist from peripheral-level tuberculosis (TB) clinics in tuberculosis-endemic countries. We conducted a clinical trial at four county-level TB clinics in China to evaluate the effectiveness of the CPA assay. TB suspects were continuously enrolled by a clinician at each clinic. Following informed consent, each patient provided two sputum specimens (spot and morning sputum). Sputum samples were tested by smear microscopy, solid culture and CPA. The National TB reference laboratory (NTRL) collected all culture positive strains and performed 16S–23S rDNA internal transcribed spacer (ITS) sequence analysis for strain identification. Solid culture was used as the gold standard to evaluate the effectiveness of CPA in detecting MTB. A total of 2200 TB-suspected patients were enrolled at the four county-level TB clinics. Compared to solid culture, the sensitivity and specificity of the CPA test for MTB detection within this group was 84.1% (95%CI, 79.5–88.6) and 97.8% (95%CI, 97.1–98.5), respectively, and the sensitivity in smear-negative cases was 59.8% (95%CI, 49.8–69.8). The test failure rate of CPA was 0.8% (32/3918), significantly lower than the 1.7% (106/6138) culture contamination rate.

1. Introduction

China has the second largest TB-burden in the world, with nearly 200,000 TB-related deaths per year [1]. TB diagnostics commonly used in Chinese TB labs are associated with a number of issues. Smear microscopy, although inexpensive, has a relatively low sensitivity, and therefore only diagnoses patients with relatively advanced disease [2]. While solid culture has a high sensitivity, it requires a turnaround time of 3–8 weeks [3]. Recently, a number of nucleic acid amplification technologies have been developed for the rapid diagnosis of MTB. Many are more sensitive than smear microscopy and demonstrate substantial advantages over conventional culture [4,5]. However, many commercialized nucleic acid tests have not found wide-spread use due to a number of remaining issues. This includes the use of complicated methodologies, the need for high-cost, high-precision equipment and disposables, and the need for highly-skilled lab technicians and associated technical support [6–8]. These tests are therefore unsuitable for use in most high TB-burden and resource-limited settings.

Cross-Priming Amplification (CPA) is a new nucleic acid amplification technique for the detection of TB and other diseases developed by Ustar Biotechnologies Co., Ltd China. Using multiple cross-linked primers, CPA can amplify a target nucleic acid under isothermal conditions without the need for any specialized equipment. The CPA testing kit for TB detection includes sample preparation (60 min), nucleic acid isothermal amplification (60 min), and hybridization and detection using a patented cross-contamination proof device (20 min) [9–11]. The detection of amplified products is performed on a lateral flow strip housed in an enclosed, sealed plastic device to prevent the leakage of amplicons.

A previous report demonstrated excellent performance of the CPA test kit at a reference laboratory [12]. In this previous study, two cross primers (double crossing CPA) targeting the *gyr*B gene were used to detect MTB in clinical samples. The CPA test detected

* Corresponding author. Center for Tuberculosis Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China.
** Corresponding author.
E-mail addresses: changtingliu@sohu.com (C. Liu), zhaoyanlin@chinatb.org (Y. Zhao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tube.2014.04.006

92.8% of pulmonary tuberculosis cases, including 87.5% of those with smear-negative disease. Here we evaluate an optimized CPA reaction that utilizes only one cross primer (single crossing CPA) targeting the IS6110 region of the MTB genome and is multiplexed with an internal amplification control reaction. As the estimated cost of CPA is 4$–5$ per test, it is suitable to be used in resource-limited settings. Our goal was to evaluate the performance of the single crossing CPA test for TB diagnosis at four peripheral labs in China and to explore its potential use as a screening tool at microscopy labs throughout China.

2. Methods

2.1. Study design

During January, 2012, NTRL staff provided a week-long training on solid culture and CPA test for the staff members from four county-level clinics in Zhengding County, Changqing County, Xinmi District, and Changping District. Lab technicians were required to take a proficiency test and only those who passed the test were allowed to participate in this study. A pilot study on the use of CPA was conducted and supervised by the NTRL at each local site for 1 month at which point performance was verified. From March, 2012 to October, 2012, the four county-level TB clinics enrolled patients suspected of having TB based on their clinical symptoms; and following informed consent, collected 2 sputum samples (spot sputum and morning sputum). Lab technicians tested each sputum sample using smear microscopy, solid culture and CPA tests. A patient was deemed positive if one sample tested positive.

NTRL staff collected all culture positive samples and performed 16S–23S rDNA internal transcribed spacer sequence analysis. Positive specimens with culture results that were contaminated, had no strain identification results or were non-TB were excluded from final analysis. The remaining samples were used to evaluate the performance of the CPA test in detecting MTB compared to solid culture among new TB suspects. We assessed the operational feasibility of the CPA test by examining indicators such as test failure rate, turnaround time and the variation of performance between sites. We also administered a user-acceptability questionnaire to establish the minimal training needs and ease of use for the CPA test.

2.2. Procedures

2.2.1. Smear microscopy

Smear microscopy was performed in accordance with the China NTP—Sputum Smear SOP and Quality Assurance Manual [13].

2.2.2. Solid culture

2 ml of sputum sample was transferred into a sealable tube with a screw cap. Depending on the viscosity of sample, 1–2 volumes of 4% sodium hydroxide pretreatment solution was added, and the sample was then vortexed for 30–60 s until it was fully mixed after which it was left to stand for 15 min. The solution (0.1 ml) was inoculated into two Lowenstein–Jensen Medium slants, which were then incubated for 24 h at 37 °C in a standing position and then cultivated further at 37 °C. Bacilli growth was monitored and recorded at Day 3 and Day 7, and then every week until the 8th week.

2.2.3. CPA

Sputum was mixed with 2–3 volumes of 4% sodium hydroxide solution and allowed to stand for 20–30 min at room temperature until fully liquefied. One ml of liquefied sputum was transferred into a 1.5 ml centrifuge tube and centrifuged at 10,000 rpm for 10 min. The pellet was suspended in 1 ml of physiological saline (0.9% w/v NaCl) and washed two times by centrifugation under the above mentioned protocol. The liquification and centrifugation for sample processing was performed in a bio-safety cabinet. An aliquot of 40 μL of a DNA extraction solution (Ustar) was transferred into the centrifuge tube containing the pellet, the tube was then placed in a boiling water bath (95–100 °C) for 10 min, and then removed and allowed to cool to room temperature. The tube was centrifuged at 10,000 rpm for 5 min and the supernatant was saved as the amplification template. 15 μL of re-suspension buffer was added to each amplification tube containing Ustar's proprietary glassified reagents followed by 20 μL of paraffin oil [14–16]. Tubes were allowed to incubate at room temperature for 2–3 min to allow the glassified reagents to dissolve completely. For the negative and positive controls 4 μL ddH_2O or supplied positive control was added to a reconstituted assay tube and 4 μL of each extracted sample was added to the remaining tubes. All reaction tubes were centrifuged again at 4000 rpm for 3–5 s before being placed on a heat block at 63 °C for 60 min. Following amplification, the reaction tubes were placed into the cartridge of the detection device, which was folded to close and then inserted into the device. The handle was closed shut to lock producing a clear "click" sound. After 15–30 min the lateral flow strip results were read by visual inspection from within the device (Figure 1). Results were determined by observing the presence or absence of a test and control line which indicate the amplification of TB DNA and internal control DNA respectively. When both test and control lines were present the sample was deemed positive. When only the control line was present the sample was deemed negative. When the control line was not observed the sample was deemed invalid, the kit maybe damaged, incorrect operation or expired. Signal intensity of the test line was compared to an intensity score-card provided by the manufacturer.

2.2.4. 16S–23S rDNA ITS sequence

In order to identify MTB and Non-tuberculousis Mycobacterium (NTM) [17], 16S–23S rDNA ITS sequence analysis was performed using a forward primer, 5′-GGCCTAACCCTCGGGAGGGAG-3′, and reverse primer 5′-CCCGAGGCATATCGCAGCCTC-3′. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Direct sequencing was performed on the ABI 3730 DNA automated sequence analyzer. Sequence results were uploaded to the NCBI website for blast analysis.

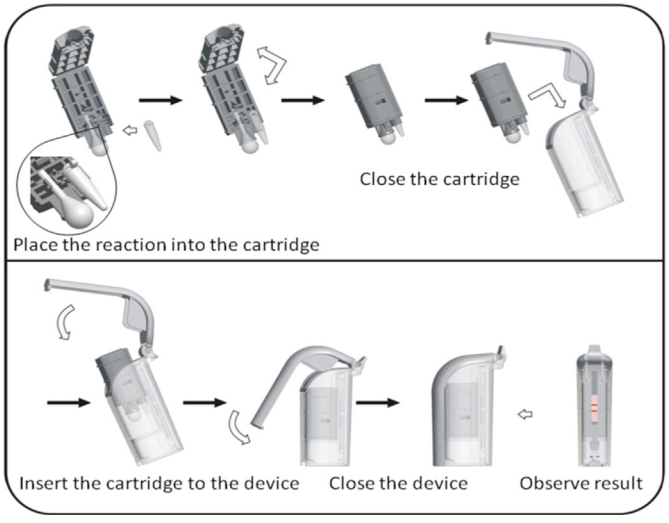

Figure 1. Process illustration for the CPA cartridge.

2.3. Statistical analysis

Results were analyzed using SPSS 17.0 software. 95% confidence intervals (CI) were calculated according to method described by Robert Newcombe [18].

2.4. Ethics statement

The study was approved by the Tuberculosis Research Ethics Review Committee of the China CDC. Written informed consent was obtained from each participant. All the authors vouch for the completeness and accuracy of the data presented.

3. Results

3.1. Smear, culture and CPA test results

From March, 2012 to October, 2012, a total of 2200 TB suspects were enrolled from the four TB clinics. Two cases had no smear results, four had contaminated culture result and one case generated an invalid CPA result. The smear positive rate was 8.1% (178/2198), the culture positive rate was 13.5% (296/2196) and the CPA positive rate was 11.9% (261/2199) (Table 1).

Correlation analysis between smear and culture results was performed on 2194 cases, excluding the 2 cases with no smear results and 4 cases with culture contamination. Among seven patients with scanty smear positive results, 6 were culture positive; and an additional 8 patients had smear positive (≥1+) results but were culture negative. Statistical analysis demonstrated that sputum smear positivity was significantly correlated with culture positivity ($p < 0.01$) (Table 2).

Among the 2197 cases with valid sputum smear and CPA results, sputum smear positivity was not related to CPA detection rates for MTB ($p > 0.05$). Of the 2019 smear-negative samples, 95 were CPA positive; and of the 7 smear scanty positive samples, 6 were CPA positive (Table 3).

3.2. Results of 16S–23S rDNA ITS sequence analysis for culture positive strains

16S–23S ITS sequence analysis was performed for 296 culture positive samples. 28 samples reported no sequencing results, 64% (18/28) samples recorded less than 10 cultured colonies. Of the 268 samples with successful sequencing results, 94.4% (253/268) were identified as MTB, 0.4% (1/268) identified as Nocardia and 5.2% (14/268) were identified as other NTMs.

Among the 14 NTM infected patients, 5 were smear positive, and 2 were CPA positive. The 2 CPA positive cases were infected with *Mycobacterium intracellulare* and were both culture and smear positive.

Table 2
The correlation between smear and culture results.

Smear	Culture						
	NEG	Scanty	1+	2+	3+	4+	Total
NEG	1889	53	38	17	10	9	2016
Scanty	1	1	2	1	1	1	7
1+	4	3	1	5	10	9	32
2+	2	1	3	15	11	14	46
3+	1	0	1	5	18	20	45
4+	1	1	0	0	9	37	48
Total	1898	59	45	43	59	90	2194

Notes: NEG = Negative.

Table 3
The correlation between smear and CPA results.

Smear	CPA		Total
	NEG	POS	
NEG	1924	95	2019
Scanty	1	6	7
1+	3	29	32
2+	3	43	46
3+	3	42	45
4+	2	46	48
Total	1936	261	2197

Notes: POS = Positive. NEG = Negative.

3.3. Quality control of solid culture and CPA assay

A total of 4402 sputum samples were cultured in 4 labs. Of the 8804 culture media tubes, 110 were contaminated, for a total contamination rate of 1.25%. This was within the SOP requirement of 5%. The culture contamination rate of each lab was also within normal ranges. A total of 178 smear positive patients were enrolled, of which 9 were culture negative, the smear positive culture negative rate was 5.1%, which was below the SOP requirement of 10%. CPA tests were performed on 3918 sputum samples, among which 32 test failures were observed, with a failure rate of 0.8% (Table 4).

3.4. Effectiveness analysis of CPA assay compared to solid culture as the gold standard

Of the 2200 new TB suspects enrolled by the four TB clinics, 2150 cases were eligible for CPA effectiveness analysis. Of the 2150 cases, 251 were culture positive and 1899 were culture negative (Figure 2).

Within this group of cases, the sensitivity and specificity of the CPA test for detecting MTB compared to solid culture was 84.1% and 97.8%, respectively. No significant difference was observed for the sensitivity of the CPA test among the four labs ($P > 0.05$); while significant differences were observed in specificity ($P < 0.01$).

Table 1
Compiled results from smear, culture and CPA for 2200 recruited patients.

	Smear				Culture				CPA			
	POS	NEG	No result	Total	POS	NEG	CON	Total	POS	NEG	Failure	Total
Zhengding	39	449	0	488	72	413	3	488	62	426	0	488
Changqing	34	448	2	484	41	442	1	484	35	449	0	484
Xinmi	60	624	0	684	92	592	0	684	79	604	1	684
Changping	45	499	0	544	91	453	0	544	85	459	0	544
Total	178	2020	2	2200	296	1900	4	2200	261	1938	1	2200

Notes: POS = Positive. NEG = Negative. CON = Contamination.

Table 4
lQC of culture and CPA at four sites.

	Cases	The rate of smear positive culture negative	Culture contamination rate	CPA failure rate
Zhengding	488	5.1%	3.3%	2.7%
Changqing	484	2.9%	2.0%	0.0%
Xinmi	684	6.7%	0.1%	0.4%
Changping	544	4.4%	0.3%	0.0%
Total	2200	5.1%	1.3%	0.8%

Among the 1982 smear-negative patients, the sensitivity and specificity of CPA test was 59.8% and 98.0% for detecting MTB (Table 5). No statistically significant difference was observed for the sensitivity of the CPA test among the four labs ($P > 0.05$), while significant differences were found in specificity ($P < 0.01$) (Table 5).

3.5. Analysis of cases whose culture result was inconsistent with the CPA result

Among the 42 culture negative but CPA positive cases, 4 were smear positive and clinically diagnosed with TB, 24 were smear and culture negative but clinically diagnosed with TB, and 14 were smear, culture and clinically negative for TB.

Among the 40 culture positive CPA negative cases, 38 were smear-negative, and 21 had a colony number of less than 10 (53%).

3.6. Comparison of turnaround time

After collecting sputum samples, the four labs conducted smear microscopy, solid culture and CPA tests on the same day. Samples that were not tested were stored at −20 °C and tested the next day. During the study, the average turnaround time of the CPA test (starting from sample treatment to test results read-out) was around 3 h, while that of solid culture was 52.5 days (56 days maximum and 7 days minimum).

3.7. Analysis of acceptability of the CPA test among lab technicians

Six laboratory operators completed a questionnaire on the acceptability of the CPA test. According to the survey, 5 (83%) believed that the CPA procedure was easier to learn and perform, 3 (50%) believed the quality control of the CPA test was equivalent to that of solid culture, 2 (33%) believed the CPA test results were more reliable compared to solid culture, 6 (100%) believed CPA had a lower failure rate compared to solid culture, 3 (50%) believed that a longer manual operation was required by solid culture compared to CPA and all believed that solid culture was associated with higher risks for lab technicians.

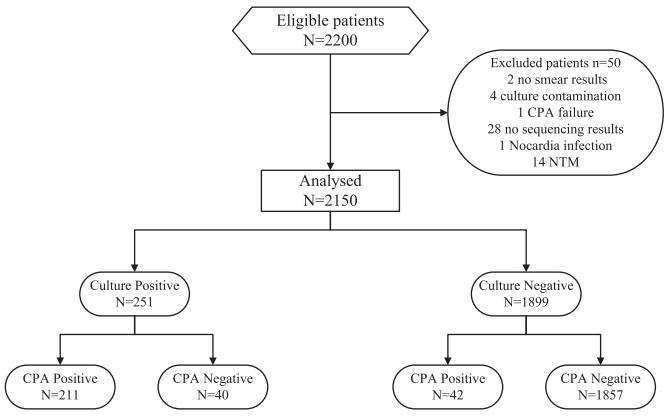

Figure 2. Case enrollment and test procedure.

4. Discussion

Early diagnosis is of vital significance to TB control. This study selected four county-level TB clinics and enrolled new TB suspects to explore the applicability and feasibility of the CPA test. The selected study sites routinely perform sputum smear microscopy for TB diagnosis and have not previously performed any molecular diagnostic testing. However, solid culture was previously performed for a research project for The Nation Survey of Drug-Resistant Tuberculosis and the labs were renovated in accordance with bio-safety requirements. Additionally a one month solid culture trial period was conducted prior to our study to ensure the quality of solid culture.

The results of this study demonstrate that CPA provided an easy to read TB result that significantly reduced the reporting time compared with solid culture. We further demonstrated that CPA can be performed by lab technicians with no prior molecular testing experience, following a short training period.

Positivity grades of smear and culture results are effective indicators of the amount of MTB. Smear and culture positivity grades were correlated in this study and it was hypothesized that smear positivity would correlate with CPA detection rates, with lower detection rates found at lower grades of smear positivity. However, our analysis found no correlation between the CPA positive detection rates and smear positivity. This is likely due to the high sensitivity of the CPA assay compared to microscopy as CPA is able to amplify as little as 4 copies of genomic DNA in less than an hour with a high degree of specificity.

Among the 14 cases with NTM infection, 2 infected with *M. intracellulare* were CPA positive. This may have been due to MTB co-infection (not confirmed) or to a lack of specificity of the CPA primers. The sensitivity and specificity of CPA for detecting MTB was 84.1% and 97.8%, lower than the 92.8% and 98.8% sensitivity and specificity reported by a previous study performed at a reference laboratory [12]. This may be explained by the modified CPA assay used in this study, which included an internal amplification control, or by differences in testing facilities and patient populations found at each research site. Among smear-negative patients, CPA was 59.8% sensitive and 98.0% specific for detecting MTB, demonstrating an improved diagnostic effectiveness among smear-negative patients in the absence of culture. Among the 42 culture negative but CPA positive patients, 14 were clinically diagnosed non-TB. These false positives may have been due to contamination during amplification or the presence of killed bacteria. Among the 40 culture positive but CPA negative patients, 21 (53%) scored a colony number of less than 10, suggesting that following sample preparation, some samples were below the CPA detection limit. Inhibitor of CPA such as hemoglobin in bloody sputum samples may have also been a problem, although theoretically the internal amplification control should have controlled for this.

Effectiveness indicators for the CPA test in the four labs were generally comparable, however, due to variations in lab capacity and skill levels, some differences were observed in the performance. We found no significant difference in the sensitivity of the CPA test among the four labs, but observed significant differences in specificity. The positive predictive values of Changping and Zhengding were 76.3% and 77.1%, respectively, lower than the other two sites. Operation bias, leading to contamination or nonspecific amplification may have contributed to these lower values. The performance of solid culture, which was used as the gold standard for this research, may have also affected the evaluation of the CPA

Table 5
Sensitivity and specificity of the CPA test from TB suspects, as compared with solid culture and strain identification.

	CPA	Culture		Sub-total	Sensitivity (95%CI)		Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
		POS	NEG		C+ (95%CI)	S-C+ (95%CI)			
Zhengding	POS	45	14	59	84.9% (75.3–94.5)	55.6% (32.6–78.5)	96.6% (94.9–98.4)	76.3% (65.4–87.1)	98% (96.7–99.4)
	NEG	8	399	407					
	Sub-total	53	413	466					
Changqing	POS	34	0	34	89.5% (79.7–99.2)	57.1% (20.5–93.8)	100% (99.3–100)	100% (39.8–100)	99.1% (98.2–99.9)
	NEG	4	440	444					
	Sub-total	38	440	478					
Xinmi	POS	68	9	77	87.2% (79.8–94.6)	61.5% (42.8–80.2)	98.5% (97.5–99.5)	88.3% (81.1–95.5)	98.3% (97.3–99.4)
	NEG	10	584	594					
	Sub-total	78	593	671					
Changping	POS	64	19	83	78.1% (69.1–87.0)	61% (46.0–75.9)	95.8% (94.0–97.7)	77.1% (68.1–86.2)	96% (94.2–97.8)
	NEG	18	434	452					
	Sub-total	82	453	535					
Total	POS	211	42	253	84.1% (79.5–88.6)	59.8% (49.8–69.8)	97.8% (97.1–98.5)	83.4% (78.8–88.0)	97.9% (97.2–98.5)
	NEG	40	1857	1897					
	Sub-total	251	1899	2150					

Notes: POS = Positive. NEG = Negative. PPV = Positive predictive value. NPV = Negative predictive value.

test. We found that the culture contamination rate was very low in Xinmi and Changping, potentially due to harsh specimen decontamination procedures. This may have decreased the sensitivity of solid culture which can only detect live bacteria. Given equal sensitivity and specificity, lower prevalence leads to lower positive predictive values. A sensitivity and specificity of 83.7% and 97.9%, and an assumed 25% prevalence, gives a positive predictive value of 93%. Given the accuracy of the CPA test and the importance of preventing patient injury due to false positives we suggest that the CPA test should be recommended for use in populations with a TB prevalence above 25%.

The LAMP TB test kit by Eiken Chemical Co., Ltd. is another promising isothermal amplification diagnostic for TB [19,20]. The first generation of this test displayed a sensitivity of 48.8% in smear-negative culture positive specimens, lower than the CPA sensitivity we report here [21]. However, the second generation PURE-LAMP kit has a reported sensitivity of 55.6% among smear-negative TB patients, similar to our CPA results. Although further implementation data is needed to fully assess this method, the PURE-LAMP kit requires fewer procedures and consumables than CPA, and the entire process from sputum sample treatment to amplification reaction and result read-out is all performed in three closed plastic devices, greatly reducing the risks of contamination. In comparison the CPA sputum processing method was fairly complicated requiring centrifugation, multiple pipetting steps and the requirement for a biological safety cabinet. Currently, Ustar is working to improve sputum sample processing and DNA extraction in order to increase sensitivity among smear-negative patients as well as usability in peripheral-level clinics. One advantage of the CPA test kit is the ability to ship all reagents at room temperature. This is achieved by a proprietary glass-transition process, developed by Ustar that glassifies the enzymes providing long-term stability at a wide range of temperatures [14–16].

In conclusion, the CPA test is a rapid, direct and reliable new diagnostic for TB detection. Through this feasibility evaluation of the CPA test, we found that this technology is well suited for use in China's microscopy labs for the screening of TB patients.

Acknowledgments

We would like to acknowledge the financial support provided by Chinese Anti-Tuberculosis Association. Thanks to the Centers for Diseases Control and Prevention (CDC) of Henan, Hebei and Shandong Province, and to the TB dispensaries of Zhengding County, Changqing County, Xinmi District, and Changping District, and to Dr. Pan Yuxuan and Dr. Wan liya for assistance with design and data analysis.

Funding: This study was supported by Major State Basic Research Development Program (2014CB744403) and Chinese Anti-tuberculosis Association. NTRL led study design, training, study coordination and monitoring, data analysis, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Global tuberculosis control — epidemiology, strategy, financing: WHO report. Geneva: World Health Organization; 2009 (WHO/HTM/TB/2009.411).
[2] Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. Expert Rev Anti Infect Ther 2007;5:327–31.
[3] Small PM, Pai M. Tuberculosis diagnosis-time for a game change. N Engl J Med 2010;363:1070–1.
[4] Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part II. Active tuberculosis and drug resistance. Expert Rev Mol Diagn 2006;6:423–32.
[5] Roetzer A, Diel R, Kohl TA, Rückert C, Nübel U, Blom J, Wirth T, Jaenicke S, Schuback S, Rüsch-Gerdes S, Supply P, Kalinowski J, Niemann S. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. PLoS Med 2013;10:e1001387. http://dx.doi.org/10.1371/journal.pmed.1001387.
[6] Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, Fukasawa Y, Yoshimura T, Miyagi C, Goto S. Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and Gen-Probe amplified Mycobacterium tuberculosis direct test. J Clin Microbiol 1993;31:3270–4.
[7] Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NT, Jones-López EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of ondemand, near-patient technology. J Clin Microbiol 2010;48:229–37.
[8] Guo Y, Zhou Y, Wang C, Zhu L, Wang S, Li Q, Jiang G, Zhao B, Huang H, Yu H, Xing W, Mitchelson K, Cheng J, Zhao Y. Rapid, accurate determination of multidrug resistance in M. tuberculosis isolates and sputum using a biochip system. Int J Tuberc Lung Dis 2009;13:914–20.
[9] Xu Gaolian, Hu Lin, Zhong Huayan. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci Rep 2012;2:246. http://dx.doi.org/10.1038/srep00246 [Epub ahead of print].

[10] Chow WH, McCloskey C, Tong Y, Hu L, You Q, Kelly CP, Kong H, Tang YW, Tang W. Application of isothermal helicase-dependent amplification with a disposable detection device in a simple sensitive stool test for toxigenic Clostridium difficile. J Mol Diagn 2008;10:453–8.
[11] Kong H, Ranalli T, Lemieux B. New isothermal molecular diagnostics platforms. IVD Technol 2007;13:35–43.
[12] Fang R, Li X, Hu L, You Q, Li J, Wu J, Xu P, Zhong H, Luo Y, Mei J, Gao Q. Cross-priming amplification for rapid detection of Mycobacterium tuberculosis in sputum specimens. J Clin Microbiol 2009;47:845–7.
[13] Ministry of Health. Disease control and prevention bureat, center for disease control and prevention. China TB control program. Smear microscopy SOP and quality control manual. Beijing: Peking Union Medical College Press; 2009.
[14] Franks f. Long-term stabilization of biologicals. Biotechnology 1994;12:253–6.
[15] Poole PH, Grande T, Angell CA. Polymorphic phase transitions in liquids and glasses. Science 1997;275:322–4.
[16] Alcock R, Cottingham MG, Rollier CS, Furze J, De Costa SD, Hanlon M, Spencer AJ, Honeycutt JD, Wyllie DH, Gilbert SC, Bregu M, Hill AV. Long-term thermostabilization of live poxviral and adenoviral vaccine vectors at supra-physiological temperatures in carbohydrate glass. Sci Transl Med 2010;2(19). http://dx.doi.org/10.1126/scitranslmed.3000490.
[17] Roth A, Fischer M, Hamid ME, Michalke S, Ludwig W, Mauch H. Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. J Clin Microbiol 1998;36:139–47.
[18] Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 1998;17:857–72.
[19] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000;15:E63.
[20] Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, Sanga E, Hoelscher M, Notomi T, Hase T, Perkins MD. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy laboratory of developing countries. J Clin Microbiol 2007;45:1936–40.
[21] Mitarai S, Okumura M, Toyota E, Yoshiyama T, Aono A, Sejimo A, Azuma Y, Sugahara K, Nagasawa T, Nagayama N, Yamane A, Yano R, Kokuto H, Morimoto K, Ueyama M, Kubota M, Yi R, Ogata H, Kudoh S, Mori T. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis 2011;15:1211–7.