Publication List

TB-LAMP

EIKEN CHEMICAL CO.,LTD.

Human
Diagnostics Worldwide

Publication list for TB-LAMP

Application in remote settings

Detecting Mycobacterium tuberculosis using the loop-mediated isothermal amplification test in South Africa

S. Reddy, S. Ntoyanto, Y. Sakadavan, T. Reddy, S. Mahomed, M. Dlamini, B. Spooner, G. Ramjee, A. Coutsoudis, N. Ngomane, K. Naidoo, K. Mlisana, P. Kiepiela

– INT J TUBERC LUNG DIS 21(10):1154–1160–

Abstract

Setting

In South Africa, KwaZulu-Natal is the epicentre of the human immunodeficiency virus (HIV) epidemic, where approximately 70% of people with tuberculosis (TB) are co-infected with HIV. Undiagnosed TB contributes to high mortality in HIV-infected patients. Delays in diagnosing TB and treatment initiation result in prolonged transmission and increased infectiousness.

Objective

To evaluate the Loopamp™ MTBC Detection kit (TB-LAMP; based on the loop-mediated isothermal amplification assay), smear microscopy and Xpert test with the gold standard of mycobacterial culture.

Methods

Sputum samples were collected from 705 patients with symptoms of pulmonary TB attending a primary health care clinic.

Results

The TB-LAMP assay had significantly higher sensitivity than smear microscopy (72.6% vs. 45.4%, P, 0.001), whereas specificity was slightly lower (99% vs. 96.8%, P¼0.05), but significantly higher than Xpert (92.9%, P¼0.004). There was no significant difference in sensitivity of smear-positive, culture-positive and smear-negative, culture-positive sputum samples using TB-LAMP vs. Xpert (respectively 95.9%/55.9% vs. 97.6%/66.1%; P ¼0.65, P ¼ 0.27). The positive predictive value of TB-LAMP was significantly higher than that of Xpert (87.5% vs. 77.0%; P ¼ 0.02), but similar to that of smear microscopy (94.2%; P ¼ 0.18). The negative predictive value was respectively 91.9%, 92.5% (P ¼ 0.73) and 83.1% (P ¼ 0.0001).

Conclusion

Given its ease of operability, the TB-LAMP assay could be implemented as a point-of-care test in primary health care settings, and contribute to reducing treatment waiting times and TB prevalence.

Comparative Evaluation of the Loop-Mediated Isothermal Amplification Assay for Detecting Pulmonary Tuberculosis

Chang-Ki Kim, Eun A Cho, Dong Mi Shin, Sung Won Choi and So Youn Shin

– Ann Lab Med 2018;38:119-124–

Abstract

Background

Early detection of tuberculosis (TB) is challenging in resource-poor settings because of limited accessibility to molecular diagnostics. The aim of this study was to evaluate the performance of the loop-mediated isothermal amplification kit (TB-LAMP) for TB diagnosis compared with conventional and molecular tests.

Methods

A total of 290 consecutive sputum samples were collected from May till September, 2015. All samples were processed using the N-Acetyl-L-cysteine (NALC) NaOH method and tested by smear microscopy, solid and liquid culture, real-time PCR, and TB-LAMP.

Results

The sensitivity of TB-LAMP for smear-positive and smear-negative samples with culture positivity was 92.0% and 58.8%, respectively. TB-LAMP was positive in 14.9% of TB culture-negative samples; however, all those samples were also positive by real-time PCR. In addition, none of the samples positive for nontuberculous mycobacteria by culture were positive by TB-LAMP. The overall agreement between TB-LAMP and real-time PCR was good; however, the concordance rate was significantly lower for real-time PCR positive samples with Ct values of 30–35.

Conclusions

TB-LAMP could replace smear microscopy and increase TB diagnostic capacity when Xpert MTB/RIF is not feasible because of poor infrastructure.

Performance of loop-mediated isothermal amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda

Lydia Nakiyingi, Prossy Nakanwagi, Jessica Briggs, Tifu Agaba, Frank Mubiru, Mark Mugenyi, Willy Ssengooba, Moses L. Joloba and Yukari C. Manabe

– BMC Infectious Diseases (2018) 18:87–

Abstract

Background

The Smear microscopy lacks sensitivity especially in HIV co-infection, resulting in undiagnosed tuberculosis (TB) and high mortality. The loop-mediated isothermal amplification (TB-LAMP) assay can be staged with minimal infrastructure, is rapid, low cost and detection can be with the naked eye. We assessed feasibility and performance of Eiken TB-LAMP test at point-of-need in TB diagnosis in a high prevalence TB/HIV rural setting in Uganda.

Methods

From October 2013-February 2014, TB-LAMP testing was performed on sputum specimens from outpatient presumptive TB adults at a district hospital and two low-level health centers in Kiboga District where smear microscopy is the available routine diagnostic option. TB-LAMP was performed by a technician after a week of training in the district hospital. The technician had no prior experience in the technology. Samples from the low-level health centers were transported to the district hospital for TB-LAMP.

Results

Of the 233 presumptive TB (126 at hospital); 113 (48.5%) were HIV-infected; 129 (55%) male; median age 40 (IQR 30-53). Compared to MTB culture, overall sensitivity and specificity of TB-LAMP were 55.4% (95 CI 44.1-66.3) and 98.0% (95 CI 94.3-99.6) respectively. Among HIV-infected participants, TB-LAMP sensitivity and specificity were 52.3% (95 CI 36.7-67.5%) and 97.1% (95 CI 89.9-99.6) respectively; and 24.4% (95% CI 12.9-39.5) and 98.6% (95% CI 95.1-99.8) respectively among smear-negatives. TB-LAMP sensitivity and specificity were 62.2% (95% CI 44.8-77.5) and 97.8% (95% CI 92.1-99.7) in the hospital setting where central testing occurred compared to 50.0% (95% CI 34.9-65.1) and 98.4% (95% CI 91.2-100) respectively in low-level health centers where specimens were transported centrally.

Conclusions

In this high prevalence TB/HIV rural setting, TB-LAMP performs better than conventional smear microscopy in diagnosis of MTB among presumptive TB patients although the sensitivity is lower than that reported by the World Health Organization. TB-LAMP can easily be performed following a short training period and in absence of sophisticated infrastructure and expertise.

Comparative study of Loopamp™ *Mycobacterium tuberculosis* Complex Kit for Rapid Detection of *Mycobacterium tuberculosis* Complex in Cameroon

Valerie Flore Donkeng Donfack, Laure Ngando, Eric Walter Yone Pefura, Dieudonné Shubesi Che, Ghislaine Ateba, Jean Joel Rim Bigna, Jean Louis Abena Foe, Christopher Kuaban, Sara Eyangoh

– Biomed Biotechnol Res J 2018;2:46-52–

Abstract

Background

The most practical test for identifying tuberculosis (TB) in developing countries remains smear microscopy. However, due to its low sensitivity, a new point-of-care diagnostic method has been developed. The purpose of this study was to assess the performance of TB-Loop-mediated isothermal amplification (TB-LAMP) test on sputum samples of suspected TB cases.

Methods

Suspected pulmonary TB patients (527) from Jamot Hospital and without any history of anti-TB treatment were consecutively included in the study. Smear microscopy, TB-LAMP, GeneXpert® MTB/RIF, and liquid culture using BACTEC 960 Mycobacteria Growth Indicator Tube (MGIT) were performed on sputum samples collected from these patients. The sensitivity and specificity of TB-LAMP were compared with smear microscopy and GeneXpert® MTB/RIF. MGIT culture was the gold standard.

Results

TB-LAMP and smear microscopy showed sensitivities of 82.6% (95% confidence interval [CI], 76.9–87.2) and 53.6% (95% CI, 46.8–60.3), respectively, and specificities of 96.0% (95% CI, 93.2–97.7) and 99.0% (95% CI, 97.1–99.7), respectively. The sensitivity and specificity of TB-LAMP were similar to GeneXpert®, (89.9%; 95% CI, 85.0–93.3 and 97.0%; 95% CI, 94.4–98.4).

Conclusions

TB-LAMP is more sensitive than currently used microscopy. It presents a favorable diagnostic tool for TB in peripheral laboratories with limited equipment, such as those in developing countries.

Comparison of TB-LAMP, GeneXpert MTB/RIF and culture for diagnosis of pulmonary tuberculosis in The Gambia

Adama L. Bojang, Francis S. Mendy, Leopold D. Tientcheu, Jacob Otu, Martin Antonio, Beate Kampmann, Schadrac Agbla, Jayne S. Sutherland

– J Infect; 72(3): 332-337–

Abstract

Background

Diagnosis of tuberculosis (TB) remains difficult, particularly in resource-limited settings. The development of nucleic acid-based tests for detection of *Mycobacterium tuberculosis* complex (MTBC) has significantly increased sensitivity compared to conventional smear microscopy and provides results within a matter of hours compared to weeks for the current gold-standard, liquid culture.

Methods

In this study we performed side-by-side comparison of mycobacterial detection assays on sputum samples from 285 subjects presenting with symptoms suggestive of TB in The Gambia and a cross-sectional cohort of 156 confirmed TB patients with a median of 2 months of treatment. A novel assay, Loop-Mediated Amplification test for TB (TB-LAMP), was compared to smear microscopy, MGIT culture and GeneXpert MTB/RIF for all samples.

Results

When culture was used as the reference standard, we found an overall sensitivity for TB-LAMP of 99% (95% CI: 94.5-99.8) and specificity of 94% (95% CI: 89.3-96.7). When latent class analysis was performed, TB-LAMP had 98.6% (95% CI: 95.9-100) sensitivity and 99% (95% CI: 98.2-100) specificity compared to 91.1% (95% CI: 86.1-96) sensitivity and 100% (95% CI: 98.2-100) specificity for MGIT culture. GeneXpert had the highest sensitivity 99.1% (95% CI: 97.1-100) but the lowest specificity 96% (95% CI: 92.6-98.3). Both TB-LAMP and GeneXpert showed high sensitivity and specificity regardless of age or strain of infection.

Conclusions

Our findings show the diagnostic utility of both GeneXpert and TB-LAMP in The Gambia. Whilst TB-LAMP requires less infrastructure, it is unable to detect drug-resistant patterns and therefore would be most suitable as a screening test for new TB cases in peripheral health clinics.

Rapid Laboratory Diagnosis of Pulmonary Tuberculosis

P. Bhirud, A. Joshi, N. Hirani, A. Chowdhary

– Int J Mycobacteriol 2017;6:296-301–

Abstract

Background

Tuberculosis (TB) ranks as the second leading cause of death from an infectious disease worldwide. Early diagnosis of Mycobacterium tuberculosis in clinical samples becomes important in the control of TB both for the treatment of patients and for curbing of disease transmission to the others in the community. The study objective was to perform Ziehl-Neelsen (ZN) staining, fluorochrome staining, line probe assay (LPA), and loop-mediated isothermal amplification (LAMP) assay for rapid detection of pulmonary TB (PTB) and to compare the results of LPA and LAMP in terms of sensitivity, specificity, and turnaround time.

Methods

A total of 891 sputum samples from clinically diagnosed/suspected cases of TB were subjected to ZN and fluorochrome staining. Smear positive samples were subjected to LPA, and smear negative were cultured on Lowenstein-Jensen media. A total of 177 samples were subjected to liquid culture and LAMP. Conventional culture was considered as "gold standard" for calculation of parameters.

Results

Light-emitting diode fluorescence microscopy had the same sensitivity as ZN with similar high specificity. LPA was performed on 548 sputum samples which includes 520 smear positive and 28 smear negative culture positive samples and multidrug-resistant TB was detected in 32.64%. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TB-LAMP on direct sputum samples was found to be 98.96%, 95%, 96%, and 98.70%, respectively, when compared with ZN smear microscopy. By considering culture as "gold standard," LAMP showed a sensitivity, specificity, PPV, and NPV of 98.94%, 96.34%, 96.90%, and 98.75%, respectively. The sensitivity and PPV of TB-LAMP were 98.97% and 96%, respectively, when compared with LPA.

Conclusions

A successful rapid laboratory diagnosis of PTB is possible when one combines the available methodology of microscopy, culture as well as molecular techniques. The LAMP assay was found to be simple, self-contained, and efficacious for early diagnosis of suspected cases of PTB with advantages of having a high throughput, no requirements of sophisticated equipment, and complex biosafety facilities.

Comparison of loop-mediated isothermal amplification assay and smear microscopy with culture for the diagnostic accuracy of tuberculosis

Baye Gelaw, Yitayal Shiferaw, Marta Alemayehu and Abate Assefa Bashaw

– BMC Infect Dis. 2017; 17: 79–

Abstract

Background

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the leading causes of death from infectious diseases worldwide. Sputum smear microscopy remains the most widely available pulmonary TB diagnostic tool particularly in resource limited settings. A highly sensitive diagnostic with minimal infrastructure, cost and training is required. Hence, we assessed the diagnostic performance of Loop-mediated isothermal amplification (LAMP) assay in detecting *M.tuberculosis* infection in sputum sample compared to LED fluorescent smear microscopy and culture.

Method

A cross-sectional study was conducted at the University of Gondar Hospital from June 01, 2015 to August 30, 2015. Pulmonary TB diagnosis using sputum LED fluorescence smear microscopy, TB-LAMP assay and culture were done. A descriptive analysis was used to determine demographic characteristics of the study participants. Analysis of sensitivity and specificity for smear microscopy and TB-LAMP compared with culture as a reference test was performed. Cohen's kappa was calculated as a measure of agreement between the tests.

Results

A total of 78 pulmonary presumptive TB patients sputum sample were analyzed. The overall sensitivity and specificity of LAMP were 75 and 98%, respectively. Among smear negative sputum samples, 33.3% sensitivity and 100% specificity of LAMP were observed. Smear microscopy showed 78.6% sensitivity and 98% specificity. LAMP and smear in series had sensitivity of 67.8% and specificity of 100%. LAMP and smear in parallel had sensitivity of 85.7% and specificity of 96%. The agreement between LAMP and fluorescent smear microscopy tests was very good ($\kappa = 0.83$, *P*-value ≤0.0001).

Conclusions

TB-LAMP showed similar specificity but a slightly lower sensitivity with LED fluorescence microscopy. The specificity of LAMP and smear microscopy in series was high. The sensitivity of LAMP was insufficient for smear negative sputum samples.

Clinical Performances of Pure TB-Lamp Kit for *M. tuberculosis* Complex Detection in Sputum Samples

Kouassi N'guessan, Jacob Adegbele, Ibrahima Coulibaly, Natacha Kouame-N'takpé, Hortense Seck-Angu, André Guei, Jacquemin Kouakou, Mireille Dosso

– J of Tuberculosis Research, 2016, 5, 129-138–

Abstract

Tuberculosis represents a main concern for public health worldwide. In poor countries, the most prevalent method for bacteriological confirmation remains Smear Sputum Microscopy (SSM). This study objective was to assess clinical performances of Loop Mediated Isothermal Amplification for TB detection (Lamp-TB). Sputum of patients presenting symptoms consistent with tuberculosis were collected according to the National Tuberculosis Control Programme guidelines in Centre Antituberculeux de Yopougon. SSM after Ziehl-Neelsen staining and TB-Lamp were blindly performed with spot sputum specimen. Samples, transported at Institut Pasteur de Cote d'Ivoire were decontaminated according to N-acetyl-L-cystein (NALC) method. In Mycobacteria Growth Indicator Tube (MGIT), 500 µl of pellet were inoculated and incubated in MGIT 960 instrument. MPT64 antigen was detected on positive culture. Of 500 patients enrolled, 469 were included. Clinical isolates of M. tuberculosis Complex were detected for 157 (33.5%). Comparatively to culture, Sensitivity and Specificity of SSM were 86% (95% Confidence interval (CI): 81% - 91%) 96% (95%IC: 94% - 98%) respectively. TB-Lamp Sensitivity was 92% (95%CI: 88% - 96%), and Specificity 94% (95%CI: 91% - 97%). Positive Predictive Value of SSM and TB-Lamp was 91.8% and 88.8% respectively. Negative Predictive Value of TB-Lamp assay was 95.7% whereas this of SSM was 93.3%. Positive Likelihood Ratio was 15.3 for TB-Lamp and 21.5 for SSM 21.5 whereas negative Likelihood of TB-Lamp was lower than SSM. Active tuberculosis was detected in162/469 (34.5%) with TB-Lamp and 147 (31.3%) with SSM. TB-Lamp assay performances estimated from sputum samples may improve detection of active TB cases in routine.

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

Lau Christen M Gray, Achilles Katamba, Pratibha Narang, Jorge Giraldo, Carlos Zamudio, Moses Joloba, Rahul Narang, CN Paramasivan, Doris Hillemann, Pamela Nabeta, Danielle Amisano, David Alland, Frank Cobelens, Catharina C Boehme

– J Clin Microbiol. 2016 Aug;54(8):1984-91–

Abstract

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.

Accuracy of LAMP-TB Method for Diagnosing Tuberculosis in Haiti

Jetsumon Taijin Kaku, Fujihiko Minamoto, Richard D'Meza, Willy Morose, Jacque Boncy5, Josette Bijou, Harry Geffrard, Miki Yoshida, and Toru Mori

– Jap J Infect Dis; 69(6): 488-492–

Abstract

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.

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

Xichao Ou, Qiang Li, Hui Xia, Yu Pang, Shengfen Wang, Bing Zhao, Yuanyuan Song, Yang Zhou, Yang Zheng, Zhijian Zhang, Zhiying Zhang, Junchen Li, Haiyan Dong, Jack Zhang, Kai Man Kam, Junying Chi, Shitong Huan, Daniel P. Chin, Yanlin Zhao

– PLoS One; 9(5): e94544–

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.

Methods

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 and smear negative 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

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.

Application in Reference Centers

Performance of the TB-LAMP diagnostic assay in reference laboratories: Results from a multicentre study

Thu Hang Pham, Jonathan Peter, Fernanda C.Q. Mello, Tommy Parraga, Nguyen Thi Ngoc Lan, Pamela Nabeta, Eloise Valli, Tatiana Caceres, Keertan Dheda, Susan E. Dorman, Doris Hillemann, Christen M. Gray, Mark D. Perkins

– *International Journal of Infectious Diseases 68 (2018) 44–49–*

Abstract

Objective

Loop To evaluate the diagnostic performance of TB-LAMP, a manual molecular tuberculosis (TB) detection method, and provide comparison to the Xpert MTB/RIF assay.

Methods

In a large multicentre study, two sputum samples were collected from participants with TB symptoms in reference laboratories in Peru, South Africa, Brazil, and Vietnam. Each sample was tested with TB-LAMP. The reference standard consisted of four direct smears, four cultures, and clinical and radiological findings. Individuals negative on conventional tests were followed up after 8 weeks. The Xpert MTB/RIF assay was performed on fresh or frozen samples as a molecular test comparison.

Results

In A total of 1036 adults with suspected TB were enrolled. Among 375 culture-confirmed TB cases with 750 sputum samples, TB-LAMP detected 75.6% (95% confidence interval (CI) 71.8–79.4%), including 97.9% (95% CI 96.4–99.4%) of smear-positive TB samples and 46.6% (95% CI 40.6–52.7%) of smear-negative TB samples. Specificity in 477 culture-negative participants not treated for TB (954 sputum samples) was 98.7% (95% CI 97.9–99.6%). TB-LAMP test results were indeterminate in 0.3% of cases.

Conclusions

Both TB-LAMP detects nearly all smear-positive and half of smear-negative TB cases and has a high specificity when performed in reference laboratories. Performance was similar to the Xpert MTB/RIF assay.

Comparison to Smear Microscopy

Detecting *Mycobacterium tuberculosis* using the loop-mediated isothermal amplification test in South Africa

S. Reddy, S. Ntoyanto, Y. Sakadavan, T. Reddy, S. Mahomed, M. Dlamini, B. Spooner, G. Ramjee, A. Coutsoudis, N. Ngomane, K. Naidoo, K. Mlisana, P. Kiepiela

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Lydia Nakiyingi, Prossy Nakanwagi, Jessica Briggs, Tifu Agaba, Frank Mubiru, Mark Mugenyi, Willy Ssengooba, Moses L. Joloba and Yukari C. Manabe

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Conclusions

In this high prevalence TB/HIV rural setting, TB-LAMP performs better than conventional smear microscopy in diagnosis of MTB among presumptive TB patients although the sensitivity is lower than that reported by the World Health Organization. TB-LAMP can easily be performed following a short training period and in absence of sophisticated infrastructure and expertise.

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Valerie Flore Donkeng Donfack, Laure Ngando, Eric Walter Yone Pefura, Dieudonné Shubesi Che, Ghislaine Ateba, Jean Joel Rim Bigna, Jean Louis Abena Foe, Christopher Kuaban, Sara Eyangoh

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Methods

Suspected pulmonary TB patients (527) from Jamot Hospital and without any history of anti-TB treatment were consecutively included in the study. Smear microscopy, TB-LAMP, GeneXpert® MTB/RIF, and liquid culture using BACTEC 960 Mycobacteria Growth Indicator Tube (MGIT) were performed on sputum samples collected from these patients. The sensitivity and specificity of TB-LAMP were compared with smear microscopy and GeneXpert® MTB/RIF. MGIT culture was the gold standard.

Results

TB-LAMP and smear microscopy showed sensitivities of 82.6% (95% confidence interval [CI], 76.9–87.2) and 53.6% (95% CI, 46.8–60.3), respectively, and specificities of 96.0% (95% CI, 93.2–97.7) and 99.0% (95% CI, 97.1–99.7), respectively. The sensitivity and specificity of TB-LAMP were similar to GeneXpert®, (89.9%; 95% CI, 85.0–93.3 and 97.0%; 95% CI, 94.4–98.4).

Conclusions

TB-LAMP is more sensitive than currently used microscopy. It presents a favorable diagnostic tool for TB in peripheral laboratories with limited equipment, such as those in developing countries.

Comparison of loop-mediated isothermal amplification assay and smear microscopy with culture for the diagnostic accuracy of tuberculosis

Baye Gelaw, Yitayal Shiferaw, Marta Alemayehu and Abate Assefa Bashaw

– BMC Infect Dis. 2017; 17: 79–

Abstract

Background

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is one of the leading causes of death from infectious diseases worldwide. Sputum smear microscopy remains the most widely available pulmonary TB diagnostic tool particularly in resource limited settings. A highly sensitive diagnostic with minimal infrastructure, cost and training is required. Hence, we assessed the diagnostic performance of Loop-mediated isothermal amplification (LAMP) assay in detecting *M.tuberculosis* infection in sputum sample compared to LED fluorescent smear microscopy and culture.

Method

A cross-sectional study was conducted at the University of Gondar Hospital from June 01, 2015 to August 30, 2015. Pulmonary TB diagnosis using sputum LED fluorescence smear microscopy, TB-LAMP assay and culture were done. A descriptive analysis was used to determine demographic characteristics of the study participants. Analysis of sensitivity and specificity for smear microscopy and TB-LAMP compared with culture as a reference test was performed. Cohen's kappa was calculated as a measure of agreement between the tests.

Results

A total of 78 pulmonary presumptive TB patients sputum sample were analyzed. The overall sensitivity and specificity of LAMP were 75 and 98%, respectively. Among smear negative sputum samples, 33.3% sensitivity and 100% specificity of LAMP were observed. Smear microscopy showed 78.6% sensitivity and 98% specificity. LAMP and smear in series had sensitivity of 67.8% and specificity of 100%. LAMP and smear in parallel had sensitivity of 85.7% and specificity of 96%. The agreement between LAMP and fluorescent smear microscopy tests was very good ($\kappa = 0.83$, *P*-value ≤0.0001).

Conclusions

TB-LAMP showed similar specificity but a slightly lower sensitivity with LED fluorescence microscopy. The specificity of LAMP and smear microscopy in series was high. The sensitivity of LAMP was insufficient for smear negative sputum samples.

Comparison to Gene Xpert/RIF

Detecting *Mycobacterium tuberculosis* using the loop-mediated isothermal amplification test in South Africa

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– INT J TUBERC LUNG DIS 21(10):1154–1160

Abstract

Setting

In South Africa, KwaZulu-Natal is the epicentre of the human immunodeficiency virus (HIV) epidemic, where approximately 70% of people with tuberculosis (TB) are co-infected with HIV. Undiagnosed TB contributes to high mortality in HIV-infected patients. Delays in diagnosing TB and treatment initiation result in prolonged transmission and increased infectiousness.

Objective

To evaluate the Loopamp™ MTBC Detection kit (TB-LAMP; based on the loop-mediated isothermal amplification assay), smear microscopy and Xpert test with the gold standard of mycobacterial culture.

Methods

Sputum samples were collected from 705 patients with symptoms of pulmonary TB attending a primary health care clinic.

Results

The TB-LAMP assay had significantly higher sensitivity than smear microscopy (72.6% vs. 45.4%, P, 0.001), whereas specificity was slightly lower (99% vs. 96.8%, P¼0.05), but significantly higher than Xpert (92.9%, P¼0.004). There was no significant difference in sensitivity of smear-positive, culture-positive and smear-negative, culture-positive sputum samples using TB-LAMP vs. Xpert (respectively 95.9%/55.9% vs. 97.6%/66.1%; P ¼0.65, P ¼ 0.27). The positive predictive value of TB-LAMP was significantly higher than that of Xpert (87.5% vs. 77.0%; P ¼ 0.02), but similar to that of smear microscopy (94.2%; P ¼ 0.18). The negative predictive value was respectively 91.9%, 92.5% (P ¼ 0.73) and 83.1% (P ¼ 0.0001).

Conclusion

Given its ease of operability, the TB-LAMP assay could be implemented as a point-of-care test in primary health care settings, and contribute to reducing treatment waiting times and TB prevalence.

Performance of the TB-LAMP diagnostic assay in reference laboratories: Results from a multicentre study

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– International Journal of Infectious Diseases 68 (2018) 44–49–

Abstract

Objective

Loop To evaluate the diagnostic performance of TB-LAMP, a manual molecular tuberculosis (TB) detection method, and provide comparison to the Xpert MTB/RIF assay.

Methods

In a large multicentre study, two sputum samples were collected from participants with TB symptoms in reference laboratories in Peru, South Africa, Brazil, and Vietnam. Each sample was tested with TB-LAMP. The reference standard consisted of four direct smears, four cultures, and clinical and radiological findings. Individuals negative on conventional tests were followed up after 8 weeks. The Xpert MTB/RIF assay was performed on fresh or frozen samples as a molecular test comparison.

Results

In A total of 1036 adults with suspected TB were enrolled. Among 375 culture-confirmed TB cases with 750 sputum samples, TB-LAMP detected 75.6% (95% confidence interval (CI) 71.8–79.4%), including 97.9% (95% CI 96.4–99.4%) of smear-positive TB samples and 46.6% (95% CI 40.6–52.7%) of smear-negative TB samples. Specificity in 477 culture-negative participants not treated for TB (954 sputum samples) was 98.7% (95% CI 97.9–99.6%). TB-LAMP test results were indeterminate in 0.3% of cases.

Conclusions

Both TB-LAMP detects nearly all smear-positive and half of smear-negative TB cases and has a high specificity when performed in reference laboratories. Performance was similar to the Xpert MTB/RIF assay.

Comparative study of Loopamp™ *Mycobacterium tuberculosis* Complex Kit for Rapid Detection of *Mycobacterium tuberculosis* Complex in Cameroon

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– Biomed Biotechnol Res J 2018;2:46-52–

Abstract

Background

The most practical test for identifying tuberculosis (TB) in developing countries remains smear microscopy. However, due to its low sensitivity, a new point-of-care diagnostic method has been developed. The purpose of this study was to assess the performance of TB-Loop-mediated isothermal amplification (TB-LAMP) test on sputum samples of suspected TB cases.

Methods

Suspected pulmonary TB patients (527) from Jamot Hospital and without any history of anti-TB treatment were consecutively included in the study. Smear microscopy, TB-LAMP, GeneXpert® MTB/RIF, and liquid culture using BACTEC 960 Mycobacteria Growth Indicator Tube (MGIT) were performed on sputum samples collected from these patients. The sensitivity and specificity of TB-LAMP were compared with smear microscopy and GeneXpert® MTB/RIF. MGIT culture was the gold standard.

Results

TB-LAMP and smear microscopy showed sensitivities of 82.6% (95% confidence interval [CI], 76.9–87.2) and 53.6% (95% CI, 46.8–60.3), respectively, and specificities of 96.0% (95% CI, 93.2–97.7) and 99.0% (95% CI, 97.1–99.7), respectively. The sensitivity and specificity of TB-LAMP were similar to GeneXpert®, (89.9%; 95% CI, 85.0–93.3 and 97.0%; 95% CI, 94.4–98.4).

Conclusions

TB-LAMP is more sensitive than currently used microscopy. It presents a favorable diagnostic tool for TB in peripheral laboratories with limited equipment, such as those in developing countries.

Comparison of TB-LAMP, GeneXpert MTB/RIF and culture for diagnosis of pulmonary tuberculosis in The Gambia

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– J Infect; 72(3): 332-337–

Abstract

Background

Diagnosis of tuberculosis (TB) remains difficult, particularly in resource-limited settings. The development of nucleic acid-based tests for detection of *Mycobacterium tuberculosis* complex (MTBC) has significantly increased sensitivity compared to conventional smear microscopy and provides results within a matter of hours compared to weeks for the current gold-standard, liquid culture.

Methods

In this study we performed side-by-side comparison of mycobacterial detection assays on sputum samples from 285 subjects presenting with symptoms suggestive of TB in The Gambia and a cross-sectional cohort of 156 confirmed TB patients with a median of 2 months of treatment. A novel assay, Loop-Mediated Amplification test for TB (TB-LAMP), was compared to smear microscopy, MGIT culture and GeneXpert MTB/RIF for all samples.

Results

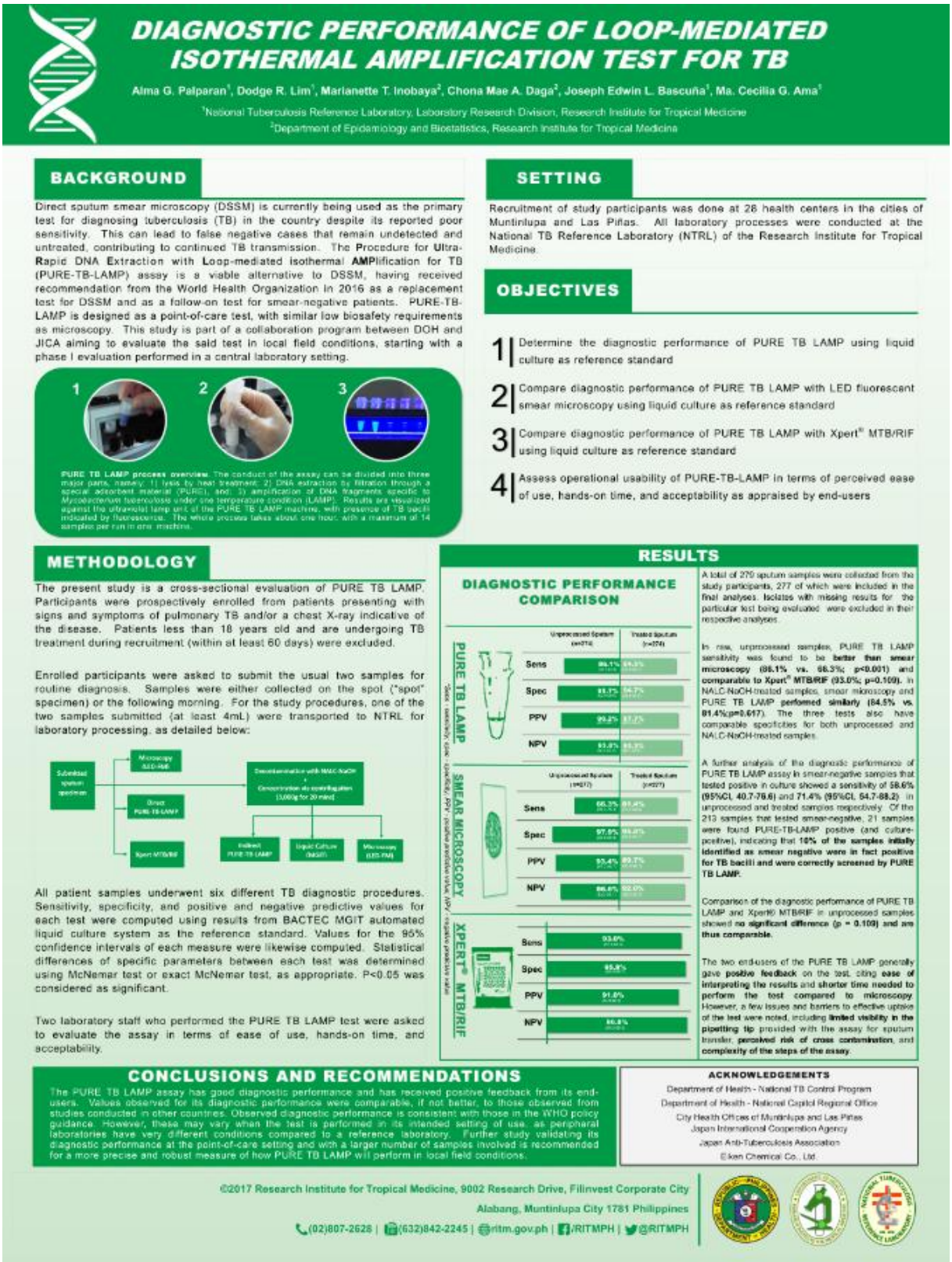
When culture was used as the reference standard, we found an overall sensitivity for TB-LAMP of 99% (95% CI: 94.5-99.8) and specificity of 94% (95% CI: 89.3-96.7). When latent class analysis was performed, TB-LAMP had 98.6% (95% CI: 95.9-100) sensitivity and 99% (95% CI: 98.2-100) specificity compared to 91.1% (95% CI: 86.1-96) sensitivity and 100% (95% CI: 98.2-100) specificity for MGIT culture. GeneXpert had the highest sensitivity 99.1% (95% CI: 97.1-100) but the lowest specificity 96% (95% CI: 92.6-98.3). Both TB-LAMP and GeneXpert showed high sensitivity and specificity regardless of age or strain of infection.

Conclusions

Our findings show the diagnostic utility of both GeneXpert and TB-LAMP in The Gambia. Whilst TB-LAMP requires less infrastructure, it is unable to detect drug-resistant patterns and therefore would be most suitable as a screening test for new TB cases in peripheral health clinics.

General

Diagnostic Performance of Loop-mediated Isothermal Amplification test for TB



DIAGNOSTIC PERFORMANCE OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION TEST FOR TB

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BACKGROUND

Direct sputum smear microscopy (DSSM) is currently being used as the primary test for diagnosing tuberculosis (TB) in the country despite its reported poor sensitivity. This can lead to false negative cases that remain undetected and untreated, contributing to continued TB transmission. The Procedure for Ultra-Rapid DNA Extraction with Loop-mediated isothermal AMPlification for TB (PURE-TB-LAMP) assay is a viable alternative to DSSM, having received recommendation from the World Health Organization in 2016 as a replacement test for DSSM and as a follow-on test for smear-negative patients. PURE-TB-LAMP is designed as a point-of-care test, with similar low biosafety requirements as microscopy. This study is part of a collaboration program between DOH and JICA aiming to evaluate the said test in local field conditions, starting with a phase I evaluation performed in a central laboratory setting.

1 2 3

PURE TB LAMP process overview. The conduct of the assay can be divided into three major parts, namely: 1) lysis by heat treatment; 2) DNA extraction by filtration through a special adsorbent material (PURE); and, 3) amplification of DNA fragments specific to *Mycobacterium tuberculosis* under one temperature condition (LAMP). Results are visualized against the ultraviolet lamp unit of the PURE TB LAMP machine, with presence of TB bacilli indicated by fluorescence. The whole process takes about one hour, with a maximum of 14 samples per run in one machine.

SETTING

Recruitment of study participants was done at 28 health centers in the cities of Muntinlupa and Las Piñas. All laboratory processes were conducted at the National TB Reference Laboratory (NTRL) of the Research Institute for Tropical Medicine.

OBJECTIVES

1. Determine the diagnostic performance of PURE TB LAMP using liquid culture as reference standard
2. Compare diagnostic performance of PURE TB LAMP with LED fluorescent smear microscopy using liquid culture as reference standard
3. Compare diagnostic performance of PURE TB LAMP with Xpert® MTB/RIF using liquid culture as reference standard
4. Assess operational usability of PURE-TB-LAMP in terms of perceived ease of use, hands-on time, and acceptability as appraised by end-users

METHODOLOGY

The present study is a cross-sectional evaluation of PURE TB LAMP. Participants were prospectively enrolled from patients presenting with signs and symptoms of pulmonary TB and/or a chest X-ray indicative of the disease. Patients less than 18 years old and are undergoing TB treatment during recruitment (within at least 60 days) were excluded.

Enrolled participants were asked to submit the usual two samples for routine diagnosis. Samples were either collected on the spot ("spot" specimen) or the following morning. For the study procedures, one of the two samples submitted (at least 4mL) were transported to NTRL for laboratory processing, as detailed below:

All patient samples underwent six different TB diagnostic procedures. Sensitivity, specificity, and positive and negative predictive values for each test were computed using results from BACTEC MGIT automated liquid culture system as the reference standard. Values for the 95% confidence intervals of each measure were likewise computed. Statistical differences of specific parameters between each test was determined using McNemar test or exact McNemar test, as appropriate. P<0.05 was considered as significant.

Two laboratory staff who performed the PURE TB LAMP test were asked to evaluate the assay in terms of ease of use, hands-on time, and acceptability.

RESULTS

DIAGNOSTIC PERFORMANCE COMPARISON

A total of 279 sputum samples were collected from the study participants, 277 of which were included in the final analyses. Isolates with missing results for the particular test being evaluated were excluded in their respective analyses.

In raw, unprocessed samples, PURE TB LAMP sensitivity was found to be **better than smear microscopy (86.1% vs. 66.3%; p<0.001)** and **comparable to Xpert® MTB/RIF (93.0%; p=0.109)**. In NALC-NaOH-treated samples, smear microscopy and PURE TB LAMP **performed similarly (84.5% vs. 81.4%;p=0.617)**. The three tests also have comparable specificities for both unprocessed and NALC-NaOH-treated samples.

A further analysis of the diagnostic performance of PURE TB LAMP assay in smear-negative samples that tested positive in culture showed a sensitivity of **58.6% (95%CI, 40.7-76.6)** and **71.4% (95%CI, 54.7-88.2)** in unprocessed and treated samples respectively. Of the 213 samples that tested smear-negative, 21 samples were found PURE-TB-LAMP positive (and culture-positive), indicating that **10% of the samples initially identified as smear negative were in fact positive for TB bacilli and were correctly screened by PURE TB LAMP.**

Comparison of the diagnostic performance of PURE TB LAMP and Xpert® MTB/RIF in unprocessed samples showed **no significant difference (p = 0.109) and are thus comparable.**

The two end-users of the PURE TB LAMP generally gave **positive feedback** on the test, citing **ease of interpreting the results** and **shorter time needed to perform the test compared to microscopy**. However, a few issues and barriers to effective uptake of the test were noted, including **limited visibility in the pipetting tip** provided with the assay for sputum transfer, **perceived risk of cross contamination**, and **complexity of the steps of the assay.**

CONCLUSIONS AND RECOMMENDATIONS

The PURE TB LAMP assay has good diagnostic performance and has received positive feedback from its end-users. Values observed for its diagnostic performance were comparable, if not better, to those observed from studies conducted in other countries. Observed diagnostic performance is consistent with those in the WHO policy guidance. However, these may vary when the test is performed in its intended setting of use, as peripheral laboratories have very different conditions compared to a reference laboratory. Further study validating its diagnostic performance at the point-of-care setting and with a larger number of samples involved is recommended for a more precise and robust measure of how PURE TB LAMP will perform in local field conditions.

ACKNOWLEDGEMENTS

Department of Health - National TB Control Program
Department of Health - National Capital Regional Office
City Health Offices of Muntinlupa and Las Piñas
Japan International Cooperation Agency
Japan Anti-Tuberculosis Association
Eiken Chemical Co., Ltd.

Inclusion of TB diagnostics on the WHO Essential Diagnostics List

C. Gilpin, K. Weyer

– INT J TUBERC LUNG DIS 22(8):827–828–

As ON 16 MAY 2018, the World Health Organization (WHO) published its first Model List of Essential In Vitro Diagnostics (EDL), a catalogue of the tests needed to diagnose the most common medical conditions as well as selected global priority diseases, including tuberculosis (TB), the leading single infectiouscause of mortality worldwide. The EDL focuses on diagnostics that 'satisfy the priority health care needs of the population and which are selected with due regard to disease prevalence and public health relevance, evidence of efficacy and accuracy, and comparative cost-effectiveness, similar to the definition of an essential medicine.' It contains 113 products used in human specimens such as blood, urine and sputum: 58 tests are listed for detection and diagnosis of a wide range of common conditions, providing an essential package for screening and management of patients. The remaining 55 tests are designed for the detection, diagnosis and monitoring of selected priority diseases such as TB, the human immunodeficiency virus, malaria, hepatitis B and C, human papillomavirus and syphilis. The EDL was developed following extensive consultation within the WHO and externally. The draft list was then reviewed by the recently established WHO Strategic Advisory Group of Experts on In Vitro Diagnostics (SAGE-IVD). Specifically, the general laboratory diagnostics were compiled based on existing WHO guidelines and technical manuals and priority medical devices lists. The technologies specific to the diagnosis of TB are referred to in the EDL, with links to the respective guidelines. These commercial IVDs include molecular line probe assays (LPAs) for the detection of resistance to first- and second-line anti-tuberculosis medicines (Hain Lifesciences, Germany; Nipro Corporation, Japan), Xpert MTB/RIF and Xpert MTB/RIF Ultra for the detection of TB and rifampicin resistance (Cepheid, Sunnyvale, CA, USA), interferon-gamma release assays (IGRAs) (Qiagen, Valencia, CA, USA; Oxford Immunotec, Oxford, UK) and the tuberculin skin test (TST) for the diagnosis of latent tuberculous infection, the lateral flow lipoarabinomannin assay (LF-LAM) to assist in the diagnosis of TB in seriously ill human immunodeficiency virus positive individuals (Alere, Waltham, MA, USA), loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary TB (Eiken Chemical Co, Tokyo, Japan), the automated liquid culture and drug susceptibility testing MGIT system (BD, Franklin Lakes, CA, USA) and light emitting diode (LED) fluorescence microscopy.

For the development of guidelines on TB diagnostics, the WHO uses the international GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach to assess the quality of evidence and to develop and report recommendations. TB diagnostics currently recommended by the WHO comprise relatively unique and complex technologies, developed almost exclusively by singlesource manufacturers. These technologies are applicable to specific patient populations and require dedicated levels of laboratory infrastructure, biosafety and technical training. In vitro results should be used to guide appropriate treatment, especially for drugresistant TB. The latest WHO policies for TB diagnosis, treatment and care have therefore been consolidated into a concise compendium. The WHO End TB Strategy calls for universal access to testing and treatment for TB, including drug-resistant forms of the disease. The EDL serves as a guiding reference for countries to update or develop their own lists of essential diagnostics based on their local context and disease epidemiology. Ensuring that WHO-recommended TB diagnostics are included in national EDLs is an important first step on the path to reaching universal coverage. Moreover, ensuring that the required laboratory infrastructure, high-quality laboratory supplies and training of laboratory workers to accurately conduct TB testing are included in adequately budgeted plans for national laboratory services is imperative for scale-up and to truly benefit patients.

Rapid molecular assays for detection of tuberculosis

Rkia Eddabra and Hassan Ait Benhassou

– Pneumonia (2018) 10:4–

Abstract

Tuberculosis (TB) is an infectious disease that remains an important public health problem at the global level. It is one of the main causes of morbidity and mortality, due to the emergence of antibiotic resistant *Mycobacterium* strains and HIV co-infection. Over the past decade, important progress has been made for better control of the disease. While microscopy and culture continue to be indispensible for laboratory diagnosis of tuberculosis, the range of several molecular diagnostic tests, including the nucleic acid amplification test (NAAT) and whole-genome sequencing (WGS), have expanded tremendously. They are becoming more accessible not only for detection and identification of *Mycobacterium tuberculosis* complex in clinical specimens, but now extend to diagnosing multidrugresistant strains. Molecular diagnostic tests provide timely results useful for high-quality patient care, low contamination risk, and ease of performance and speed. This review focuses on the current diagnostic tests in use, including emerging technologies used for detection of tuberculosis in clinical specimens. The sensitivity and specificity of these tests have also been taken into consideration.

Commercial products to preserve specimens for tuberculosis diagnosis: a systematic review

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– INT J TUBERC LUNG DIS 22(7):741–753–

Abstract

Setting

Eliminating tuberculosis in high-burden settings requires improved diagnostic capacity. Important tests such as Xpert MTB/RIF and culture are often performed at centralised laboratories that are geographically distant from the point of specimen collection. Preserving specimen integrity during transportation, which could affect test performance, is challenging.

Objective

To conduct a systematic review of commercial products for specimen preservation for a World Health Organization technical consultation.

Design

Databases were searched up to January 2018. Methodological quality was assessed using Quality Assessment of Technical Studies, a new technical study quality-appraisal tool, and Quality Assessment of Diagnostic Accuracy Studies. Studies were analysed descriptively in terms of the different products, study designs and diagnostic strategies used.

Results

Four products were identified from 16 studies: PrimeStore-Molecular-Transport-Medium (PS-MTM), FTA card, GENO_CARD (all for nucleic acid amplification tests [NAATs]) and OMNIgene_SPUTUM (OMS; culture, NAATs). PS-MTM, but not FTA card or GENO_CARD, rendered *Mycobacterium tuberculosis* non-culturable. OMS reduced Löwenstein-Jensen but not MGIT™ 960™ contamination, led to delayed MGIT time-to-positivity, resulted in Xpert performance similar to cold chain-transported untreated specimens, and obviated the need for N-acetyl-L-cysteine-sodium hydroxide decontamination. Data from paucibacillary specimens were limited. Evidence that a cold chain improves culture was mixed and absent for Xpert. The effect of the product alone could be discerned in only four studies.

Conclusions

Limited evidence suggests that transport products result in test performance comparable to that seen in cold chain-transported specimens.

Point of care diagnostics for tuberculosis

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– Pulmonol. 2018;24(2):73---85–

Abstract

The goals of the End TB strategy, which aims to achieve a 90% reduction in tuberculosis (TB) incidence and a 95% reduction in TB mortality by 2035, will not be achieved without new tools to fight TB. These include improved point of care (POC) diagnostic tests that are meant to be delivered at the most decentralised levels of care where the patients make the initial contact with the health system, as well as within the community. These tests should be able to be performed on an easily accessible sample and provide results in a timely manner, allowing a quick treatment turnaround time of a few minutes or hours (in a single clinical encounter), hence avoiding patient loss-to-follow-up. There have been exciting developments in recent years, including the WHO endorsement of Xpert MTB/RIF, Xpert MTB/RIF Ultra, loop-mediated isothermal amplification (TB-LAMP) and lateral flow lipoarabinomannan (LAM). However, these tests have limitations that must be overcome before they can be optimally applied at the POC. Furthermore, worrying short- to medium-term gaps exist in the POC diagnostic test development pipeline. Thus, not only is better implementation of existing tools and algorithms needed, but new research is required to develop new POC tests that allow the TB community to truly make an impact and find the ‘‘missed TB cases’’.

Evaluation of the Analytical Performance of the PURE-TB-LAMP Assay for Tuberculosis Detection

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– Medical Research Archives; Assay for tuberculosis detection; Volume 1; Issue 2–

Abstract

Given the prevalence and lethality of tuberculosis (TB) in developing countries, there is an ongoing need for rapid, simple, and low-cost detection method that nonetheless sensitive and highly specific. The present study evaluated the basic performance of a novel TB detection method combining procedure for ultra-rapid extraction (PURE) and loop-mediated isothermal amplification for TB (TB-LAMP). The PURE-TB-LAMP assay detected four *Mycobacterium tuberculosis* complexes and did not show any cross-reactivity with 18 species of non-TB mycobacteria (NTM) or with 10 species of other bacteria that cause respiratory tract infections such as Streptococcus pneumonia, underscoring its high specificity for TB detection. The analytical sensitivity of the assay was 100 CFU/ml for M. tuberculosis strain H37Rv cell and was unaffected by the presence of excess amounts of *M. avium* cells (a typical NTM species) or human genomic DNA. Moreover, when used with a range of artificial specimens prepared by spiking known amounts of cultured M. tuberculosis cells, the PURE method efficiently removed various inhibitory materials from a variety of samples such as sputum, urine, simulated gastric fluid, and whole blood, demonstrating the applicability of this assay to these samples. These results suggest that the PURE-TB-LAMP assay is a highly effective and accessible TB detection method that can be useful in resource-limited communities.

Loop-mediated isothermal amplification as alternative to PCR for the diagnosis of extra-pulmonary tuberculosis

Hopkins D. Joon, M. Nimesh,† D. Saluja

– J Clin Microbiol. 2016 Aug;54(8):1984-91–

Abstract

Background

The main challenge in combatting extra-pulmonary tuberculosis (EPTB) is the lack of a rapid, reliable and inexpensive diagnostic test for the detection of *Mycobacterium tuberculosis*.

Objective

To evaluate the diagnostic potential of an L-serine dehydratase gene (sdaA) loop-mediated isothermal amplification (LAMP) assay for the detection of M. tuberculosis in clinical specimens from presumptive EPTB patients.

Methods

An in-house sdaA LAMP assay was used to analyse clinical specimens (n = 315) for the diagnosis of EPTB compared with culture and the composite reference standard (CRS) comprising culture and polymerase chain reaction (PCR) using insertion sequence (IS) 6110 and mpb64 as target genes.

Results

The sdaA LAMP assay showed the highest sensitivity (93.3%) in comparison to culture; the sensitivity of IS6110 PCR, mpb64 and sdaA PCR assay was respectively 80%, 86.7% and 90%. In comparison to CRS, the LAMP assay had a sensitivity of 92.5% and a specificity of 99.2%, with a high positive (121.11) and a low negative likelihood ratio (0.08).

Conclusions

Due to its speed, simplicity, sensitivity and specificity, the sdaA LAMP assay is a potential diagnostic test for the diagnosis of EPTB, particularly in resource-limited settings.

Loop-mediated isothermal amplification of DNA

Notomi T1, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T

– Nucleic Acids Res; 28(12): e63–

Abstract

We have developed a novel method, termed loop-mediated isothermal amplification (LAMP), that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions. This method employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. An inner primer containing sequences of the sense and antisense strands of the target DNA initiates LAMP. The following strand displacement DNA synthesis primed by an outer primer releases a single-stranded DNA. This serves as template for DNA synthesis primed by the second inner and outer primers that hybridize to the other end of the target, which produces a stem-loop DNA structure. In subsequent LAMP cycling one inner primer hybridizes to the loop on the product and initiates displacement DNA synthesis, yielding the original stem-loop DNA and a new stem-loop DNA with a stem twice as long. The cycling reaction continues with accumulation of 10(9) copies of target in less than an hour. The final products are stem-loop DNAs with several inverted repeats of the target and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target in the same strand. Because LAMP recognizes the target by six distinct sequences initially and by four distinct sequences afterwards, it is expected to amplify the target sequence with high selectivity.

Unitaid Tuberculosis – Diagnostics Technology Landscape. 5th edition

Unitaid

– www.unitaid.org (28.08.2017)–

Parasite There are currently some products that have been marketed for several years with an intended use in peripheral settings. However, of these only one technology has been sufficiently evaluated in a variety of settings in order to now be approved by WHO, the Loopamp™ MTBC Detection kit from Eiken Chemical Corp. (Japan).6 Since 2012, this test has undergone 20 evaluation studies in 17 countries. This is the first NAAT product specifically designed for use in microscopy-level facilities to receive WHO endorsement, which had two conditional recommendations for the use of the assay instead of conventional SSM where patients present with symptoms of TB or as a follow-on test where follow-on testing of conventional SSM results is necessary.
The first version of the assay was released in 2011 by the company and has undergone some modifications since in terms of specimen and reaction volumes and the protocol used for the assay. The assay is well suited to resource-limited settings as the equipment is relatively simple and several user steps are added to reduce instrumentation complexity, including sample preparation and the interpretation of test results (Figure 17). The assays can be batched and up to 14 samples can be screened in a single run with control reactions. The assay uses 60 µL of raw sputum that is transferred to the sample preparation tube. MTB cells are inactivated and lysed via a combination exposure to highly alkaline conditions and temperature (90 °C) for 5 minutes. The sample preparation tube interlocks with a sample neutralization tube where the pH of the heated sample is neutralized. The final step is the addition of an applicator tube whereby ~30 µL of the liquid treated sputum contents can be expressed into a reaction tube. The assay reagents are stored as a glassified pellet inside each reaction tube lid. The DNA extracts are added to the tube strip, the caps are then closed and the tube strips inverted for 2 minutes to wet the reagents and then mixed to permit their introduction to the samples. The assay uses LAMP, an isothermal manual DNA amplification method that takes 40 minutes to perform at 67 °C in this assay. After incubation, the reactions are terminated by briefly heating at an elevated temperature. The results of each reaction are scored visually by the user via fluorescence, which is generated when DNA is amplified by the TB-LAMP reaction. The light source is supplied with the instrument and the user compares the green fluorescence of the positive control to each test. A negative control provides further user input to score any negative tests. The summary data from the 2016 WHO policy guidance document noted the pooled sensitivity of TB-LAMP was higher than for SSM, ranging from 77.7% to 80.3%. The pooled sensitivity for the TB-LAMP among SSM positive patients ranged from 95.2% to 96.6% across studies, depending on the reference standard used.6 The pooled specificity of the assays was also slightly variant depending on the reference methods used to qualify results with ranges from 97.7% to 98.1%. Eiken Chemical Corp. has partnered with HUMAN Diagnostics Worldwide (Germany) to globally distribute and market the assay and instrumentation necessary to perform the Loopamp™ MTBC Detection kit. Training requirements for LAMP is similar to the amount of training for smear microscopy.175 These products have been available since Q4 2016 and eligible countries and negotiated pricing can be accessed on the FIND website.20 The Loopamp™ Pure DNA Extraction kit costs €298.20 (90 extractions), the Loopamp™ MTB Detection Kit is €352.50 (2 x 48 reactions) and the instrument is €2450.00.

Analytical sensitivity and specificity of a loop-mediated isothermal amplification (LAMP) kit prototype for detection of *Trypanosoma cruzi* DNA in human blood samples

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– *PLoS Negl Trop Dis. 2017 Jul 20;11(7):e0005779*–

Abstract

In This study aimed to assess analytical parameters of a prototype LAMP kit that was designed for detection of Trypanosoma cruzi DNA in human blood. The prototype is based on the amplification of the highly repetitive satellite sequence of T.cruzi in microtubes containing dried reagents on the inside of the caps. The reaction is carried out at 65°C during 40 minutes. Calcein allows direct detection of amplified products with the naked eye. Inclusivity and selectivity were tested in purified DNA from Trypanosoma cruzi stocks belonging to the six discrete typing units (DTUs), in DNA from other protozoan parasites and in human DNA. Analytical sensitivity was estimated in serial dilutions of DNA samples from Sylvio X10 (Tc I) and CL Brener (Tc VI) stocks, as well as from EDTA-treated or heparinized blood samples spiked with known amounts of cultured epimastigotes (CL Brener). LAMP sensitivity was compared after DNA extraction using commercial fiberglass columns or after "Boil & Spin" rapid preparation. Moreover, the same DNA and EDTA-blood spiked samples were subjected to standardized qPCR based on the satellite DNA sequence for comparative purposes. A panel of peripheral blood specimens belonging to Chagas disease patients, including acute, congenital, chronic and reactivated cases (N = 23), as well as seronegative controls (N = 10) were evaluated by LAMP in comparison to qPCR. LAMP was able to amplify DNAs from T. cruzi stocks representative of the six DTUs, whereas it did not amplify DNAs from Leishmania sp, T. brucei sp, T. rangeli KPN+ and KPN-, P. falciparum and non-infected human DNA. Analytical sensitivity was 1x10-2 fg/µL of both CL Brener and Sylvio X10 DNAs, whereas qPCR detected up to 1x 10-1 fg/µL of CL Brener DNA and 1 fg/µl of Sylvio X10 DNA. LAMP detected 1x10-2 parasite equivalents/mL in spiked EDTA blood and 1x10-1 par.eq/mL in spiked heparinized blood using fiberglass columns for DNA extraction, whereas qPCR detected 1x10-2 par.eq./mL in EDTA blood. Boil & Spin extraction allowed detection of 1x10-2 par.eq /mL in spiked EDTA blood and 1 par.eq/ml in heparinized blood. LAMP was able to detect T.cruzi infection in peripheral blood samples collected from well-characterised seropositive patients, including acute, congenital, chronic and reactivated Chagas disease. To our knowledge, this is the first report of a prototype LAMP kit with appropriate analytical sensitivity for diagnosis of Chagas disease patients, and potentially useful for monitoring treatment response.

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