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Evaluation of the TB-LAMP assay for the rapid diagnosis of pulmonary tuberculosis in Northern India

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SUMMARY

SETTING: A tertiary care hospital in North India.

OBJECTIVE: To evaluate a commercial kit-based loop-mediated isothermal amplification (TB-LAMP) assay for the diagnosis of pulmonary tuberculosis (PTB).

DESIGN: A total of 530 patients presenting with PTB symptoms were enrolled and one sputum sample was collected from each patient. The TB-LAMP assay (Loopamp™ MTBC Detection kit) was performed on the raw sputum sample. The remaining sample was used for smear microscopy and mycobacterial culture. A cartridge-based nucleic acid amplification test (NAAT, Xpert® MTB/RIF assay) was also performed on the processed pellet.

RESULTS: The sensitivity and specificity of the TB-LAMP assay in culture-positive samples obtained from 453 patients presenting with PTB symptoms (77 specimens were excluded) were respectively 100% (95%CI 94.7–100) and 99.2% (95%CI 97.8–99.8). The sensitivity and specificity of Xpert in culture-positive samples were respectively 82.6% (95%CI 71.5–90.6) and 94.9% (95%CI 92.2–96.8). A concordance of 0.75 was obtained between the two NAATs (TB-LAMP assay and Xpert) using the κ statistic.

CONCLUSION: The TB-LAMP assay showed high sensitivity and specificity with limited requirement of testing infrastructure, and is thus a promising diagnostic tool for TB diagnosis in resource-poor settings.

KEYWORDS: nucleic acid amplification test; diagnosis; tuberculosis; Xpert® MTB/RIF; TB-LAMP

TUBERCULOSIS (TB), an infectious disease with very high morbidity and mortality, is an important public health problem. According to the World Health Organization's (WHO's) 2016 global tuberculosis report, there were an estimated 10.4 million people with active TB disease and 1.7 million TB deaths in 2015.[1] India accounts for a quarter of the global burden of TB, with 2.2 million new cases annually.[2] Rapid diagnosis of disease is essential for timely initiation of treatment, which helps in reducing transmission. In resource-poor settings, the diagnosis of TB becomes more complex due to the small number of WHO-endorsed tests and their limitations. Smear microscopy has low sensitivity (60–70%) and lacks reproducibility;[3] culture for *Mycobacterium tuberculosis* is currently the gold standard, but it is laborious and time-consuming.

Nucleic-acid amplification tests (NAAT) for TB diagnosis have very high sensitivity and specificity. However, the high costs and need for well-equipped settings and continuous power sources limit their use in resource-poor settings.[4–8] The sensitivity and specificity of the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) NAAT test on respiratory specimens is respectively 88% and 99%.[9]

Another NAAT for TB, the loop-mediated isothermal amplification (LAMP) assay, is rapid, and final results can be detected through visualisation with the naked eye, thus reducing costs and resources. LAMP is based on the isothermal nucleic acid amplification method, using either two or three sets of primers or a polymerase with high strand displacement activity in addition to replication activity.[10] The sensitivity of in-house LAMP assays for detection of the *M. tuberculosis* complex using different targets ranges from 69% to 100%, with high specificity. The major limitation of in-house LAMP is the inconsistent results from one study to the next.[11]

The TB-LAMP kit (Loopamp™ MTBC Detection kit) was developed by Eiken Chemical Company (Tokyo, Japan) and evaluated by the Foundation for Innovative New Diagnostics (FIND) in different countries.[12] Studies from Gambia[13] and China[14] showed sensitivity and specificity of TB-LAMP ranging from respectively 70% to 99% and 94% to 96%.

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Table 1 Performance of diagnostic tests for the detection of *Mycobacterium tuberculosis*

	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
TB-LAMP	100 (94.8–100)	99.2 (97.8–99.8)	95.83 (88.3–99.1)	100.00 (99–100)
Xpert® MTB/RIF	75 (63.7–84.2)	96.8 (94.5–98.34)	82.6 (71.5–90.6)	95.05 (92.4–97)

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.

The present study was part of a multi-country evaluation of TB-LAMP to determine its sensitivity and specificity in different geographical regions. The main objective of our study was to evaluate the diagnostic accuracy of TB-LAMP for the detection of *M. tuberculosis* and to compare it with that of the Xpert assay in respiratory specimens.

MATERIALS AND METHODS

Study design

Five hundred and thirty consecutive individuals with suspected pulmonary TB (PTB) (cough > 2 weeks, night sweats, fever, unintentional weight loss) attending the DOTS Centre at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, in 2014 were enrolled for this study. Sputum samples were collected or received at the DOTS Centre, PGIMER. Detailed patient information, including age, sex, human immunodeficiency virus (HIV) status and time of initiation of treatment, was collected at the time of enrolment. The paediatric age group (<12 years) and HIV-positive patients were excluded from the study.

The study protocol was approved by the Institute Ethics Committee of PGIMER (PGI/IEC/2013/2057-58). All patients provided written informed consent.

Routine microbiological assays

Smears were prepared and reported according to India's Revised National Tuberculosis Control Programme guidelines. First, 60 µl of sputum was used for the TB LAMP assay and the remainder of the sample was processed/decontaminated using the *N*-acetyl-l-cysteine-sodium hydroxide method.[15] Then, 500 µl of the decontaminated sample was inoculated into a BACTEC™ MGIT™ tube (BD, Franklin Lakes, NJ, USA) and incubated for 42 days in a MGIT™ 960™ instrument according to the manufacturer's instructions. Positive tubes were confirmed for the *M. tuberculosis* complex using SD BIOLINE TB Ag MPT64 Rapid (Standard Diagnostics, Gyeonggi-do, Republic of Korea). The remainder of the sample was stored at –20°C and transferred for Xpert.[16]

TB-LAMP assay

The TB LAMP assay was performed using Loopamp according to the manufacturer's instructions. In brief, 60 µl of sputum sample was transferred into heating tubes using a modified pipette. Heating tubes were mixed, incubated at 90°C for 5 min and fitted into an absorbent tube, then 25–35 µl of DNA was eluted from the absorbent tube and transferred into injection caps, mixed with lyophilised reagents and incubated at 67°C for 40 min. The final result of the TB LAMP assay was obtained using ultraviolet fluorescence detection.[12]

Statistical analysis

Culture of the *M. tuberculosis* complex was taken to be the gold standard for TB diagnosis. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the TB-LAMP assay and Xpert assay were calculated. Concordance between LAMP and Xpert was calculated using the κ statistic. Data were analysed using Medcalc (Ostend, Belgium; https://www.medcalc.org/)

RESULTS

Study population

Sputum samples from 530 individuals with suspected TB were collected from February to November 2014. Of these, 77 samples were excluded due to culture contamination; in 56 of these, 5 were identified as mycobacteria other than TB, 11 were from HIV-positive persons and 5 showed as 'invalid/error' on Xpert. Of the 453 samples analysed for the study, 285 (62.9%) were from males and 168 (37.1%) from females, with age ranging from 13 to 82 years. There were 37 (8.2%) smear-positive cases, and culture was positive for the *M. tuberculosis* complex in 69 (15.2%) cases. Thirty-two (7.1%) cases were smear-positive, culture-positive, 37 (8.2%) were smear-negative, culture-positive and 5 (1.1%) were smear-positive, culture-negative. As culture is considered the gold standard, the sensitivity, specificity, PPV and NPV of the other molecular tests were calculated (Table 1).

Sensitivity and specificity of TB-LAMP and Xpert MTB/RIF

Among the 453 samples from individuals with suspected TB, TB-LAMP was positive in 72 (69 in culture-positive samples and 3 in culture-negative samples). The overall sensitivity of TB-LAMP was 100% (95%CI 94.79–100) and specificity was 99.2% (95%CI 97.76–99.84) (Table 1). In smear-positive, culture-positive and smear-negative, culture-positive samples, the sensitivity and specificity of TB-

Table 2 Concordance between the TB-LAMP assay and Xpert® MTB/RIF assay

	TB-LAMP-positive	TB-LAMP-negative	κ value	Proportion of agreement
Xpert-positive	59	18	0.75	0.93 (0.90–0.95)
Xpert-negative	13	368		

LAMP were also 100%. The PPV and NPV of TB-LAMP were respectively 95.8% (95%CI 88.3–99.1) and 100% (95%CI 99.1–100). Xpert was positive in 76 cases, 57 of which were culture-positive and 19 smear-negative. The overall sensitivity of Xpert was 75% (95%CI 63.7–84.3); 26/31 smear-positive, culture-positive cases and 31/37 smear-negative, culture-positive samples were Xpert-positive. The specificity of Xpert was 96.8% (95%CI 94.5–98.3). The PPV and NPV of Xpert were respectively 82.6% (71.6–90.7) and 95.1% (92.4–97). Agreement between the two molecular tests was 0.93 (0.90–0.95), with a κ value of 0.75 (Table 2).

DISCUSSION

The molecular diagnosis of TB is rapid and accurate, but the cost remains a challenge for resource-limited settings. The Xpert assay has shown good sensitivity and specificity for the diagnosis of TB in respiratory samples. Nevertheless, the cost of testing and the need for continuous power supplies remain major issues in resource-poor settings.

We evaluated another NAAT, the TB-LAMP assay, for the detection of *M. tuberculosis* and compared its performance with that of Xpert and MGIT culture in 453 PTB samples. The sensitivity of the TB-LAMP assay in culture-confirmed TB was 100%, which was similar to reports from the Gambia[13] and Ivory Coast,[17] which showed overall sensitivities of respectively 99% and 92%. However, the evaluation study from China showed an overall sensitivity of 70.6%, ranging from 67% to 83% at different sites.[14] When compared with culture-based reference standards, the sensitivity of the TB-LAMP test was 76–80% in the WHO's 2016 policy guidelines,[13] whereas the sensitivity of manual TB-LAMP assays has been reported to have a wide range (71–100%).[11] The specificity of the TB-LAMP assay in the WHO's policy guidelines was 97–98% when compared with culture as the reference standard; it was 94–98% in two other studies. However, in the present study, three false-positive results were detected and the specificity of the TB-LAMP assay was 99.2%.

The overall sensitivity of Xpert was 75% when culture was considered the gold standard. The sensitivity of Xpert was lower than that reported in the WHO's 2016 policy guidelines, probably because we conducted the assays on frozen samples and the turnaround time of the test was longer. The specificity of Xpert was 96.8%, which is comparable with that of previous studies.[9] The proportion of agreement between the TB-LAMP assay and Xpert was 0.93 (0.90–0.95), with a κ of 0.75, which indicates good agreement between these two molecular tests. The test failure rate for TB-LAMP and Xpert was respectively zero and five cases, which is indicative of the robustness of the TB-LAMP assay.

The WHO has recommended the TB-LAMP assay as a replacement or follow-on test for sputum smear microscopy for PTB diagnosis.[18] The main advantage of the TB-LAMP assay is its cost-effectiveness and the fact that very little volume of the sample is required, in comparison with other molecular methods. It is less sensitive to amplification inhibitors, which might be an advantage in extra-pulmonary samples, as these are often mixed with external particles. There is also a

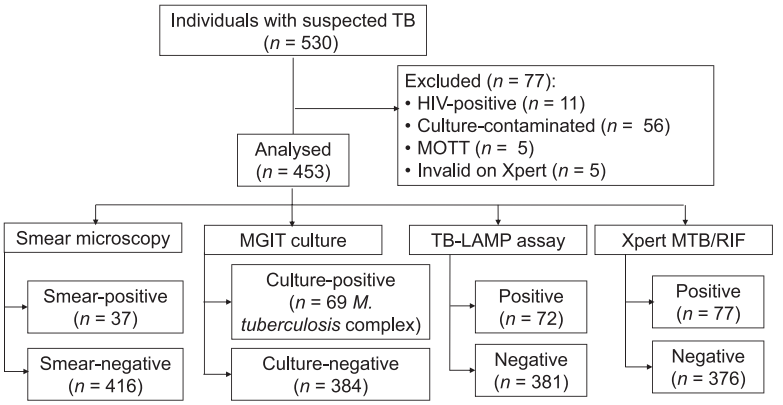

Figure Flow chart of patient enrolment and diagnostic tests. TB = tuberculosis; HIV = human immunodeficiency virus; MOTT = mycobacteria other than tuberculosis.

need to simplify the working protocol to make it as user-friendly as Xpert.

Our study had one main limitation, the small sample size. A large sample size could be beneficial for narrow CIs, which can improve the diagnostic accuracy of the test.

In conclusion, the TB-LAMP test is a new diagnostic technology that is rapid, accurate and cost-effective for the detection of *M. tuberculosis* in resource-poor settings.

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Conflicts of interest: none declared.

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RESUME

CONTEXTE : Un hôpital de niveau tertiaire dans le Nord de l'Inde.

OBJECTIF : Un test commercial en kit de méthode d'amplification isothermale facilitée par boucle (loop-mediated isothermal amplification, TB-LAMP) a été évalué dans le diagnostic de la tuberculose pulmonaire (TBP).

SCHÉMA : Un total de 530 patients se présentant avec des symptômes de TBP ont été enrôlés et un échantillon de crachats a été recueilli chez chaque patient. Le test TB-LAMP (Loopamp™ MTBC Detection Kit) a été réalisé sur l'échantillon de crachats non traité. L'échantillon restant a été utilisé pour une microscopie de frottis et une culture à la recherche de mycobactéries. Le test d'amplification de l'acide nucléique sur cartouche (test Xpert® MTB/RIF) a également été réalisé sur le pellet traité.

RÉSULTATS : Sur les 453 patients se présentant avec des symptômes de TBP (77 spécimens ont été exclus), la sensibilité et la spécificité du test TB-LAMP sur des échantillons à culture positive ont été de 100% (IC95% 94,7–100%) et 99,2% (IC95% 97,8–99,8), respectivement. La sensibilité et la spécificité de l'Xpert sur des échantillons à culture positive ont été de 82,6% (IC95% 71,5–90,6) et 94,9% (IC95% 92,2–96,8), respectivement. Une concordance de 0,75 a été obtenue entre les deux tests basés sur l'amplification de l'acide nucléique, c'est-à-dire le test TB-LAMP et l'Xpert, grâce à des statistiques κ.

CONCLUSION : Dans cette étude, le test TB-LAMP a montré une grande sensibilité et spécificité ; requérant peu d'infrastructure, il est un outil de diagnostic prometteur pour le diagnostic de la TB dans des contextes pauvres en ressources.

RESUMEN

MARCO DE REFERENCIA: Un hospital de atención terciaria en el norte de la India.

OBJETIVO: Evaluar el rendimiento diagnóstico de un estuche comercial de la prueba de amplificación isotérmica (del ADN) mediada por bucles (TB-LAMP) en los casos de tuberculosis pulmonar (TBP).

MÉTODO: Se incluyeron en estudio 530 pacientes que acudieron con síntomas de TBP y se recogió una muestra de esputo de cada paciente. Se practicó la prueba TB-LAMP (*Loopamp™ MTBC Detection Kit*) en la muestra de esputo sin tratar. El resto de la muestra se utilizó en la baciloscopia y el cultivo de micobacterias. Se aplicó además el cartucho de la prueba de amplificación de ácidos nucleicos (Xpert® MTB/RIF) con el sedimento procesado de la muestra.

RESULTADOS: En las muestras con cultivo positivo de los 453 pacientes que acudieron con síntomas de TBP (se excluyeron 77 muestras) la sensibilidad de la prueba TB-LAMP fue 100% (IC95% 94,7–100) y su especificidad fue 99,2% (IC95% 97,8–99,8). En las muestras con cultivo positivo, la sensibilidad de la prueba Xpert fue 82,6% (IC95% 71,5–90,6) y su especificidad fue 94,9% (IC95% 92,2–96,8). El coeficiente de concordancia κ de las dos pruebas de amplificación de ácidos nucleicos fue 0,75.

CONCLUSIÓN: En el presente estudio la prueba TB-LAMP exhibió alta sensibilidad y especificidad y poca exigencia en materia de infraestructura; esta prueba aparece como un instrumento promisorio en el diagnóstico de la TB en los entornos con escasos recursos.