The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis

THE END TB STRATEGY

World Health Organization

The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis

Policy guidance

WHO Library Cataloguing-in-Publication Data

The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis. Policy guidance.
1. Tuberculosis, Pulmonary - diagnosis. 2. Mycobacterium tuberculosis. 3. Nucleic Acid Amplification Techniques. 4. Guideline.

I.World Health Organization.

ISBN 978 92 4 151118 6 (NLM classification: WF 300)

Subject headings are available from WHO institutional repository

Designed by GPS Publishing

Printed in Spain

WHO/HTM/TB/2016.11

Contents

Tables

Figures

Acknowledgements

This document was prepared by Christopher Gilpin and Alexei Korobitsyn with input from Karin Weyer and Wayne van Gemert (all at WHO's Global TB Programme) on the basis of consensus agreed at a Guideline Development Group meeting convened by WHO via an online webinar on 16 January 2016.

WHO steering group

Christopher Gilpin, Karin Weyer, Alexei Korobitsyn, Wayne van Gemert and Fuad Mirzayev (all at WHO's Global TB programme)

Chair of the WHO Guidelines Development Group

Jan Brozek (McMaster University, Canada)

GRADE methodologist of the WHO Guidelines Development Group

Jan Brozek (McMaster University, Canada)

Members of the WHO Guideline Development Group

Jeremiah Chakaya (Kenya Medical Research Institute, Kenya), Gavin Churchyard (The Aurum Institute, South Africa), Daniela Maria Cirillo (HSR San Raffaele Scientific Institute/TB Supranational Reference Laboratory, Italy), Paul Klatser (Royal Tropical Institute, The Netherlands), Arata Kochi (independent consultant, Switzerland), Satoshi Mitarai (Japan Anti-Tuberculosis Association, Japan), Beatrice Mutayoba (Ministry of Health and Social Welfare, United Republic of Tanzania), Ingrid Oxley Oxland (Nelson Mandela Metropolitan University, South Africa), Thomas M. Shinnick (independent consultant, United States of America), Karen Steingart (Liverpool School of Tropical Medicine, England), Wendy Stevens (University of the Witwatersrand, South Africa), Francis Varaine (Médecins Sans Frontières, France), Anna Vassall (London School of Hygiene and Tropical Medicine, England), Yasuhiro Yasutomi (National Institutes of Biomedical Innovation, Health and Nutrition, Japan)

Systematic Review authors

Adithya Cattamanchi (lead author, systematic review; San Francisco General Hospital, University of California, San Francisco, United States), Priya B. Shete (San Francisco General Hospital, University of California, San Francisco, United States), Katherine Farr (San Francisco General Hospital, University of California, San Francisco, United States), Luke Strnad (San Francisco General Hospital, University of California, San Francisco, United States), Hojoon Sohn (lead author, economic evalution; Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Canada)

External Review Group

Kathleen England (KNCV Tuberculosis Foundation, The Netherlands), Levan Gagnidze (International Organization for Migration, Thailand), Rumina Hassan (TB Supranational Reference Laboratory Pakistan, Aga Khan University, Pakistan), Nazir Ismail (TB Supranational Reference Laboratory, National Institute of Communicable Diseases, South Africa), Richard Lumb (Adelaide TB Supranational Reference Laboratory, Australia), Enos Masini (National TB Programme, Kenya), Alaine Nyaruhirira (Management Sciences for Health, South Africa), Somsak Rienthong (Bangkok TB Supranational Reference Laboratory, Thailand), Leen Rigouts (Institute of Tropical Medicine, Belgium), Maria Alice Telles (Management Sciences for Health, Brazil)

Acknowledgement of financial support

Funding from the United States Agency for International Development is gratefully acknowledged through United States Agency for International Development–WHO Consolidated Grant No. GHA-G-00-09-00003/US 2014-741.

Abbreviations

CI	confidence interval
EVAL	evaluation
FIND	Foundation for Innovative New Diagnostics
GDG	Guideline Development Group
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HIV	human immunodeficiency virus
LAMP	loop-mediated isothermal amplification
MTBC	*Mycobacterium tuberculosis* complex
PICO	Population, Intervention, Comparator, Outcome
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RFA	request for application
TB	tuberculosis
TB-LAMP	loop-mediated isothermal amplification for detection of *Mycobacterium tuberculosis*
WHO	World Health Organization
USAID	United States Agency for International Development

Declarations and management of conflicts of interests

The members of the Guideline Development Group (GDG), the systematic review team and the External Review Group completed Declarations of Interests. These were reviewed by the WHO Steering Group prior to the meeting in January 2016. Each Declaration of Interest was assessed to determine whether an interest had been declared and, if so, whether it was insignificant or potentially significant. If the Steering Group determined that no relevant interest had been declared or such interest was insignificant or minimal, individuals were invited to participate. None of the declarations made by GDG members were determined to be significant or potentially significant. Two individuals declared significant interests and were designated as observers to the webinar. Members of the systematic review team were invited to provide technical input and answer technical questions. The observers and the authors of the systematic review did not participate in the GRADE (Grading of Recommendations Assessment, Development and Evaluation) process or in the final discussions, when recommendations were developed. Also, they were not involved in developing the report of the GDG meeting or in preparing WHO's policy guidance.

The following interests were declared:

None declared

Jan Brozek (Chair), Jeremiah Chakaya, Gavin Churchyard, Moses Joloba, Paul Klatser, Arata Kochi and Yasuhiro Yasutomi declared no conflicts of interest.

Declared, determined to be insignificant

Wendy Stevens declared that she had received remuneration for performing validations of other TB assays (from Abbott Laboratories, Alere, Cepheid, DNA Genotek, Hain Lifescience, Hoffman–LaRoche), generally in the form of reagents; she received no funding related to TB-LAMP**.**

Anna Vassall declared that she had acted as a consultant to model the cost–effectiveness of new diagnostics.

Karen Steingart declared that she had performed systematic reviews of the use of the GenoType MTBDR*sl* (Hain Lifescience) and the lateral flow urine lipoarabinomannan assay (known as LF-LAM assay).

Thomas M. Shinnick declared that he was a former employee of the United States Centers for Disease Control and Prevention (CDC). The CDC has supported his travel and research related to his work on the laboratory services needed to control tuberculosis. He declared that he had represented the CDC's positions on the laboratory services needed for tuberculosis diagnosis, treatment, and control, and served on the Data and Safety Monitoring Board organized by Otsuka Pharmaceutical for the clinical trial of delamanid. He declared that no remuneration had been received.

Daniela Maria Cirillo declared that she had received research grants from FIND (the Foundation for Innovative New Diagnostics) and the Italian government (€17 000) to evaluate a new TB test. No funding related to TB-LAMP was declared**.**

Declared, determined to be significant (observer status)

Satoshi Mitarai declared presenting at an Eiken Chemical–sponsored symposium at the 46th Union World Conference on Lung Health in Cape Town, South Africa, in December 2015. He declared that he had received research support, including paid travel to a forum on LAMP research in China.

Beatrice Mutayoba was the co-investigator for the TB-LAMP evaluation study in the United Republic of Tanzania.

Date of review: 2020 or earlier if significant additional evidence become available.

Executive summary

Background

World Health Organization's (WHO's) End TB Strategy calls for the early diagnosis of tuberculosis (TB) and for universal drug-susceptibility testing, highlighting the critical role of laboratories in the post-2015 era in rapidly and accurately detecting TB and drug resistance.[1] Molecular assays based on nucleic acid amplification techniques, such as polymerase chain reaction, have been developed for rapid TB diagnosis and are being implemented in developing countries. Loop-mediated isothermal amplification (LAMP) is a unique, temperature-independent technique for amplifying DNA that is simple to use, providing a visual display that is easy to read; additionally, the technique is robust and can be used at peripheral health centres, where microscopy is performed. LAMP methods have been used to detect malaria and several neglected tropical diseases.

A commercial molecular assay to detect *Mycobacterium tuberculosis* complex that is based on LAMP techniques (TB-LAMP) has been developed by Eiken Chemical Company (Tokyo, Japan). TB-LAMP is a manual assay that requires less than 1 hour to perform and can be read with the naked eye under ultraviolet light.

In 2012, WHO convened a Guideline Development Group (GDG) that reviewed the evidence and found that TB-LAMP technology had potential as a tool for rapid TB diagnosis, but the group recommended that additional studies be conducted.[2] Since 2012, 20 studies in 17 countries have been conducted. In January 2016, WHO convened a GDG via webinar to review the recent evidence for TB-LAMP.

Objectives, rationale and methods used to develop the guidance

TB-LAMP requires minimal laboratory infrastructure and has few biosafety requirements; and it has been evaluated for use as a rapid alternative to sputum-smear microscopy, which remains the primary diagnostic test for pulmonary TB in resource-limited settings. This document summarizes the evidence and makes recommendations about using the commercial TB-LAMP assay [the Loopamp™ *Mycobacterium tuberculosis* complex (MTBC) detection kit, Eiken Chemical Company] to detect MTBC directly from sputum specimens from persons with signs and symptoms consistent with pulmonary TB.

The objectives for developing the guideline were to determine:

- the diagnostic accuracy of TB-LAMP for detecting pulmonary TB compared with mycobacterial culture when used as a replacement test for sputum-smear microscopy among all adults including HIV-positive adults with signs and symptoms consistent with pulmonary TB;
- the diagnostic accuracy of TB-LAMP for detecting pulmonary TB compared with mycobacterial culture when used as an add-on test following negative sputum-smear microscopy among adults with signs and symptoms consistent with pulmonary TB;

---

[1] Implementing the End TB Strategy: the essentials. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.31; http://www.who.int/tb/publications/2015/end_tb_essential.pdf, accessed 15 March 2016).

[2] The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis: Expert Group meeting report. Geneva: World Health Organization; 2013 (WHO/HTM/TB/2013.05; http://apps.who.int/iris/bitstream/10665/83142/1/WHO_HTM_TB_2013.05_eng.pdf?ua=1&ua=1, accessed 15 March 2016).

- the difference in diagnostic accuracy between TB-LAMP and the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States) for detecting pulmonary TB compared with mycobacterial culture among all adults with signs and symptoms consistent with pulmonary TB;
- the proportion of indeterminate or invalid results occurring when TB-LAMP is used to detect pulmonary TB among all adults with signs and symptoms consistent with pulmonary TB.

The authors of the systematic review identified all published and unpublished studies of TB-LAMP that had been conducted since January 2012 using the modified design-locked assay protocol. Standardized eligibility criteria were applied to select individual studies and study participants for inclusion in the analysis. The quality of the included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.

WHO policy recommendations developed from the evidence synthesis process by the GDG are summarized below.

WHO's policy recommendations

1. TB-LAMP may be used as a replacement test for sputum-smear microscopy to diagnose pulmonary TB in adults with signs and symptoms consistent with TB (conditional recommendation, very low-quality evidence).
2. TB-LAMP may be used as a follow-on test to smear microscopy in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary (conditional recommendation, very low-quality evidence).

Remarks

- These recommendations apply to settings where it is possible to perform conventional sputum-smear microscopy.
- TB-LAMP should not replace the use of rapid molecular tests that detect TB and resistance to rifampicin, especially among populations at risk of multidrug-resistant TB.
- Due to the limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over sputum-smear microscopy for testing persons living with HIV who have signs and symptoms consistent with TB.
- These recommendations apply only to the use of TB-LAMP in testing sputum specimens from patients with signs and symptoms consistent with pulmonary TB.
- These recommendations are extrapolated to using TB-LAMP in children based on the generalization of data from adults, while acknowledging the difficulties of collecting sputum specimens from children.

1. Background

Tuberculosis (TB) remains a large-scale public health problem. Key global priorities for TB care and control include improving case-detection and detecting patients earlier, particularly patients with smear-negative TB disease. In 2014, only 63% (6 million) of an estimated 9.6 million people who developed TB were reported to WHO, meaning that globally 37% of the estimated cases of TB are undetected. WHO has identified the development and evaluation of new diagnostic tools as a necessary part of further efforts.[3]

WHO's End TB Strategy calls for the early diagnosis of TB and for universal drug-susceptibility testing, highlighting the critical role of laboratories in the post-2015 era in rapidly and accurately detecting TB and drug resistance.[4] Molecular assays based on nucleic acid amplification techniques, such as polymerase chain reaction, have been developed for rapid TB diagnosis and are being implemented in developing countries. A commercial molecular assay, the LoopampTM *Mycobacterium tuberculosis* complex (MTBC) detection kit (Eiken Chemical Company, Tokyo, Japan), uses loop-mediated isothermal amplification (LAMP), and is referred to as TB-LAMP. TB-LAMP is a manual assay that requires less than 1 hour to perform and can be read with the naked eye under ultraviolet light. Because of its limited infrastructure requirements and relative ease of use, TB-LAMP is being explored for use as a rapid diagnostic test that can be used as an alternative to smear microscopy in resource-limited settings. LAMP methods have been used to detect malaria and several neglected tropical diseases.

In 2012, WHO convened a Guideline Development Group (GDG) that recognized TB-LAMP offered a manual molecular approach to TB detection that could be feasibly implemented in peripheral-level microscopy laboratories once laboratory technicians had undergone adequate training.[5, 6] The advantages of TB-LAMP are that it is relatively high-throughput, does not require sophisticated instruments, and has biosafety requirements similar to those of sputum-smear microscopy.

Since 2012, 20 additional studies in 17 countries have been conducted. WHO convened a GDG meeting in January 2016 to review evidence from a systematic review and meta-analysis of data from individual participants in these studies.

The evidence reviewed and this policy guidance apply only to the use of the commercial TB-LAMP manual assay. Other DNA-based assays for detecting MTBC were not evaluated. Any new or generic assay intended to detect the presence of MTBC using LAMP or another DNA amplification method should be adequately evaluated and validated in the settings where it is intended to be used, as per WHO policy.[7]

---

[3] Global tuberculosis report 2015. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.22; http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf, accessed 15 March 2016).

[4] Implementing the End TB Strategy: the essentials. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.31; http://www.who.int/tb/publications/2015/end_tb_essential.pdf, accessed 16 March 2016).

[5] The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis: Expert Group meeting report. Geneva: World Health Organization; 2013 (WHO/HTM/TB/2013.05; http://apps.who.int/iris/bitstream/10665/83142/1/WHO_HTM_TB_2013.05_eng.pdf?ua=1&ua=1, accessed 16 August 2016).

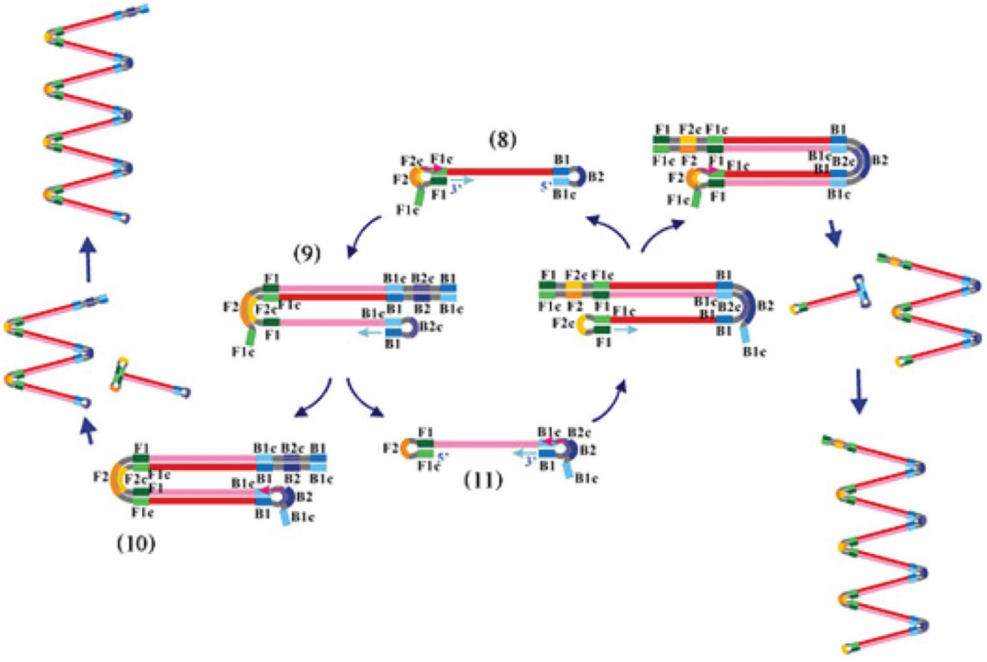
[6] Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol. 2007;45:1936–40. doi: 10.1128/JCM.02352-06.

[7] Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.11; http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf, accessed 18 August 2016). http://www.who.int/tb/publications/implementing_TB_diagnostics/en/

Index test

The fundamental amplification reaction requires four types of primers, which are complementary to six regions of the target gene (Fig. 1). As double-stranded DNA is in a condition of dynamic equilibrium at a temperature of around 65 °C, one of the LAMP primers can anneal to the complementary sequence of double-stranded target DNA, initiating DNA synthesis with the DNA polymerase; strand displacement activity displaces and releases a single-stranded DNA. Due to the complementarity of the 5'-end of the forward inner primer (known as FIP) and the backward inner primer (BIP) in nearby regions of the target amplicon, loop structures are formed. This allows variously sized structures, consisting of alternately inverted repeats of the target sequence on the same strand, to be formed in rapid succession.

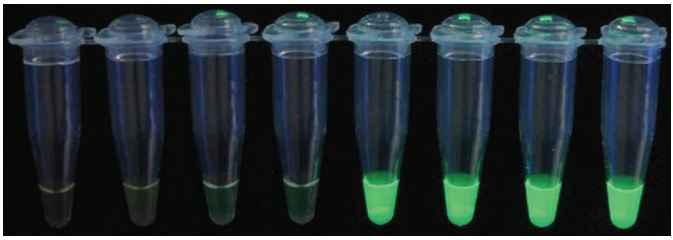
Figure 1. Molecular principles of TB-LAMP



The addition of loop primers, which contain sequences complementary to the single-stranded loop region on the 5′-end of the hairpin structure, speeds the reaction by providing a greater number of starting points for DNA synthesis. Using loop primers, amplification by 109 to 1010 times can be achieved within 15–30 minutes. The version of TB-LAMP that was evaluated includes loop primers for a total of six primers binding to eight locations. This requirement for homogeneous sequences at multiple binding sites preserves the specificity of the assay even in the absence of a probe.

The LAMP method is relatively insensitive to the accumulation of DNA and DNA by-products (pyrophosphate salts), so the reaction proceeds until large amounts of amplicon are generated. This feature makes it possible to visually detect successful amplification using double-stranded DNA-binding dyes, such as SYBR green, by detecting the turbidity caused by precipitating magnesium pyrophosphate or by using a non-inhibitory fluorescing reagent that is quenched in the presence of divalent cations. Fig. 2 shows calcein, unquenched by pyrophosphate consumption of divalent cations, fluorescing under ultraviolet light. The turbid, fluorescent product is easily seen with the naked eye.

Figure 2. Visual display of TB-LAMP results under ultraviolet light



The following steps were used for the version of TB-LAMP that was evaluated (Fig. 3).

1. Sample preparation (10–20 minutes):

- a wide-bore disposable pipette was used to collect 60 µL of sputum from a specimen container and then transfer it to a heating tube containing the extraction solution;
- this was mixed by inverting 3–4 times; the heating tube was placed into the heating block at 90 °C for 5 minutes to lyse and inactivate mycobacteria;
- the heating tube was removed from the heating block and let cool for 2 minutes;
- the heating tube was attached to an adsorbent tube and mixed by shaking until all the powder had been completely mixed with the solution;
- an injection cap was placed onto the adsorbent tube and screwed tightly to pierce the seal;
- the nozzle was inserted into a reaction tube and drops of solution (30 µl) were transferred to the reaction tube.

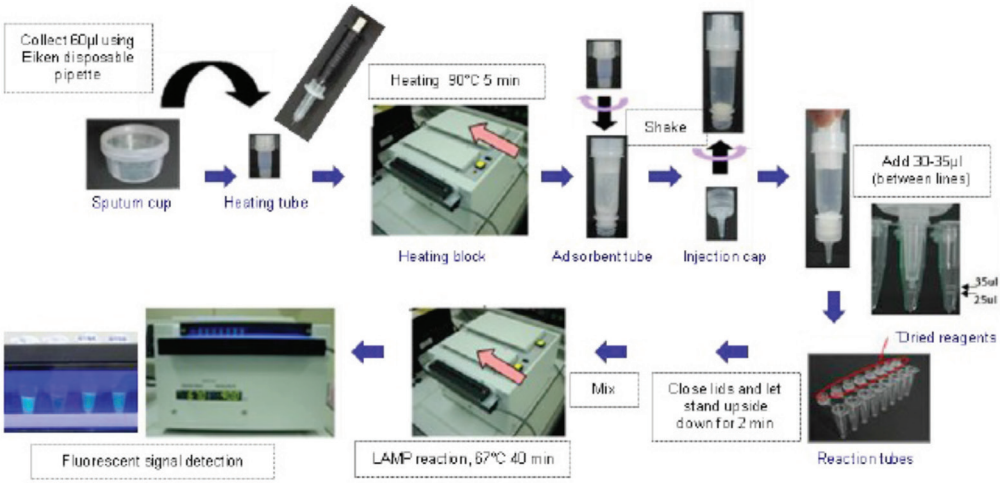
2. Amplification (40 min):

- the temperature on the digital display on the incubator was confirmed to be 67 °C;
- the reaction tubes were loaded into the heating block and the reaction was started;
- the amplification was stopped automatically after 40 minutes.

3. Visual detection of fluorescent light from the reaction tube using ultraviolet light (0.5–1 minute):

- the reaction tubes were transferred into the fluorescence detector and the results were recorded;
- the reaction tubes were discarded without being opened;
- waste was disposed of by following national guidelines.

Figure 3. Schematic description of the workflow for TB-LAMP



2. Methods

2.1. Evidence synthesis

In accordance with WHO's standards for assessing evidence when formulating policy recommendations, the GRADE approach (Grading of Recommendations Assessment, Development and Evaluation, see http://www.gradeworkinggroup.org/) was used. GRADE provides a structured framework for evaluating the accuracy of diagnostic tests and their impact on patients and public health.

The evaluation used the GRADE system to determine the quality of the evidence and provide information on the strength of the recommendations using PICO questions agreed by the GDG. PICO refers to the following four elements that should be included in questions that govern a systematic search of the evidence: the Population targeted by the action or intervention (in the case of systematic reviews of the accuracy of diagnostic tests, P is the population of interest); the Intervention (I is the index test), the Comparator (C is the comparator test or tests); and the Outcome (O is usually sensitivity and specificity). The PICO questions for the review are given below.

This systematic review addressed the following questions framed in the PICO style recommended for evidence-based medicine.

PICO questions addressed by the Guideline Development Group

1. What is the diagnostic accuracy of TB-LAMP for detecting pulmonary TB in adults when TB-LAMP is used as a replacement test for sputum-smear microscopy compared with culture as a reference standard? (Results were stratified by HIV status.)

2. What is the diagnostic accuracy of TB-LAMP for detecting pulmonary TB in adults when TB-LAMP is used as an add-on test following negative sputum-smear microscopy compared with culture as a reference standard?

3. What is the difference in diagnostic accuracy between TB-LAMP and the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States) for detecting pulmonary TB in reference to mycobacterial culture among all adults?

4. What is the proportion of indeterminate or invalid results when TB-LAMP is used to detect pulmonary TB among all adults and among HIV-positive adults?

2.2. Criteria for considering studies for this review

The review included all prospective studies that evaluated the use of TB-LAMP on sputum samples from adults with signs and symptoms consistent with pulmonary TB that were conducted in settings with an intermediate or high burden of TB. All included studies were conducted after 1 January 2012, using the final, design-locked TB-LAMP protocol that had been modified by the manufacturer. Twenty studies were identified, including all studies that were directly conducted by FIND (the Foundation for Innovative New Diagnostics) or funded through FIND following a request for applications (RFAs). To confirm that the list of studies identified was complete, Google Scholar and PubMed were searched using the terms "TB LAMP", "TB-LAMP", and "tuberculosis LAMP" (search completed on 1 October 2015).

Studies were excluded if they did not exclude patients who started TB treatment within 60 days of enrolment or if rapid speciation to confirm the presence of MTBC in positive cultures was not performed. Studies that used TB-LAMP on frozen specimens were excluded. In addition, individual study participants were excluded if they had a history of TB; were less than 18 years old; did not have results from rapid speciation testing for MTBC; or if they had a positive mycobacterial culture

but speciation testing was negative, suggesting the presence of non-tuberculous mycobacteria. Individual participants were also excluded if TB-LAMP was performed on samples other than sputum or if the total reaction volume was less than 25 µL. For the comparison of TB-LAMP with the Xpert MTB/RIF assay, individual participants were excluded if Xpert MTB/RIF was performed on frozen specimens or if valid results from both TB-LAMP and Xpert MTB/RIF were not available from the same specimen. Study participants who could not be classified as TB-positive or TB-negative based on the reference standard definitions described below were excluded.

The following mycobacterial culture reference standards were used to classify TB status. Eligible studies performed one or more sputum cultures on solid media (Löwenstein–Jensen), liquid media using the BACTEC mycobacterial growth indicator tube (MGIT; Becton Dickinson, Franklin Lakes, NJ, United States), or both. To account for the different number of cultures performed by studies and the different number of culture results available for participants, three hierarchical culture-based reference standards were used to assess diagnostic accuracy.

Standard 1 comprised

- TB: at least one positive culture confirmed to be MTBC by speciation testing
- Not TB: no positive and at least two negative cultures performed on two different sputum samples

Standard 2 comprised

- TB: at least one positive culture confirmed to be MTBC by speciation testing
- Not TB: No positive and at least two negative cultures performed on at least one sputum sample

Standard 3 comprised

- TB: at least one positive culture confirmed to be MTBC by speciation testing
- Not TB: No positive and at least one negative culture

Across the three standards, there is an expected trade-off between the yield of a confirmed TB diagnosis (highest with Standard 1 and lowest with Standard 3) and the number of studies or participants included in the analysis (lowest with Standard 1 and highest with Standard 3). Thus, using Standard 1, the potential for false-negative index test results is highest and for false-positive index test results is lowest. Also using Standard 1, the number of studies and study participants included is expected to be lowest because it excludes studies that performed only one culture and study participants for whom only one negative culture result was available due to culture contamination; in contrast, using Standard 3, the number of studies and study participants is highest.

Studies recorded TB-LAMP and Xpert MTB/RIF assay results as negative, positive, or indeterminate or invalid, in accordance with the manufacturer's recommendations. Sputum-smear microscopy varied across studies in terms of (1) the type of microscopy (the use of one or more of direct Ziehl–Neelsen stains, concentrated Ziehl–Neelsen, direct fluorescence microscopy or concentrated fluorescence microscopy), (2) the number of sputum specimens examined (one, two or three specimens), and (3) the number of smears prepared from each specimen examined (one or two smears). All studies recorded semi-quantitative microscopy results in accordance with WHO guidelines. [8] Microscopy results were standardized across studies by (1) considering only results from direct Ziehl–Neelsen staining and direct fluorescence microscopy, (2) considering only the first two smear results if more than two direct Ziehl–Neelsen or direct fluorescence microscopy results were available, and (3) defining patients to be sputum smear-positive if at least one acid-fast bacillus was seen in any sputum smear.

[8] Lumb R, Van Deun A, Bastian I, Fitz-Gerald M. Laboratory diagnosis of tuberculosis by sputum microscopy: the handbook. Adelaide: SA Pathology, Global Laboratory Initiative; 2013 (http://www.stoptb.org/wg/gli/assets/documents/TB%20MICROSCOPY%20HANDBOOK_FINAL.pdf, accessed 21 March 2016).

2.3. Data collection and analysis

For studies conducted by or funded through FIND, study protocols, inclusion criteria and definitions were provided by FIND along with data on individual participants. Further review of the individual-level data was performed to verify that the study and individual participants met eligibility criteria. For studies not affiliated with FIND, individual-level data were requested from the investigators and reviewed by at least two authors to determine whether data met the criteria for inclusion in the review.

For each study, the minimum data required for each individual enrolled were:

- Age
- Smear microscopy results (including semi-quantitative scoring)
- Culture results (positive, negative or contaminated)
- TB-LAMP results (positive, negative or invalid)
- Information about the study's workflow.

Additionally, where available, the following were also collected:

- HIV status of participants
- sample collection time (spot or morning)
- number of culture days to positivity for MGIT or quantitative scoring for Löwenstein–Jensen
- results of species identification test (MTBC or non-tuberculous mycobacteria)
- TB-LAMP final reaction volume (< 25 µl, 25–35 µl or > 35 µl)
- operator who performed TB-LAMP
- Xpert MTB/RIF test results (when performed; positive, negative, indeterminate)
- drug-susceptibility test results (conventional or line probe assay).

Data were extracted from Microsoft Excel spreadsheets or Microsoft Access, SAS entries (SAS Analytics), or EpiData entries (EpiData Association), depending on the study, and entered into standardized data columns in Microsoft Excel.

Using the GRADE framework, calculations of test sensitivity and specificity were used as proxy measures for patients' outcomes; these outcomes were based on the relative importance or impact of false-positive and false-negative results. Poor sensitivity would result in *false-negative* results so that patients with TB would not be correctly diagnosed, which would have negative consequences in terms of delaying the initiation of effective treatment, developing more severe disease, and in terms of morbidity, mortality and further transmission of disease. Poor specificity would result in false-positive results so patients without TB would be prescribed unnecessary treatment, which may increase adverse effects.

Rates for true positives, true negatives, false positives and false negatives were calculated using the likely prevalence of TB among persons suspected of having TB. Prevalences of 5% and 15% were used to cover the lower and upper levels of prevalence of TB among symptomatic persons seeking care.

The evaluation of the impact on patients was based on a balance among the following values:

- *true positives* – the benefit to patients from rapid diagnosis and treatment;
- *true negatives* – the benefit to patients who would be spared unnecessary treatment (the benefits of reassurance and alternative diagnosis);

- *false positives* – the likelihood of anxiety and morbidity caused by additional testing, unnecessary treatment and possible adverse effects; the possible stigma associated with a diagnosis of TB; and the chance that a false positive might halt further diagnostic evaluation;
- *false negatives* – the increased risk of morbidity and mortality, delayed initiation of treatment and the continued risk of TB transmission.

The sensitivity and specificity of TB-LAMP, smear microscopy and the Xpert MTB/RIF assay in each study were calculated for each mycobacterial culture–based reference standard. The proportion of indeterminate or invalid TB-LAMP results was calculated as the number of indeterminate or invalid results divided by the total number of patients eligible to be included in the analysis of TB-LAMP accuracy among all adults. For all review questions, heterogeneity was assessed visually with forest plots and statistically with I2 statistics.

2.4. Guideline Development Group meeting

Following the initial GDG meeting on TB-LAMP in 2012, a GDG meeting was convened by WHO in January 2016 to review the evidence from 20 new studies, of which 13 studies conducted in 11 countries met the inclusion criteria. The guideline methodologist participated in the initial planning and scoping and in the development of the key questions for the GDG meeting but did not participate in the meeting.

The WHO Steering Group was responsible for scoping the guideline, drafting the PICO questions and overseeing evidence retrieval and analyses. The Steering Group was also responsible for selecting the members of the GDG and the External Review Group, for managing Declarations of Interest and for organizing the GDG meetings. A brief biography of each GDG member was made available for public scrutiny on the website of the WHO Global TB Programme (http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/) 2 weeks prior to the GDG meeting. PICO questions were drafted by the Steering Group and were presented to the GDG for discussion and modification. The Steering Group also prepared an initial list of relevant outcomes, including desirable effects and undesirable effects, and requested the GDG to identify any other important outcomes.

During the meeting, the Steering Group helped the GDG formulate recommendations based on the evidence presented. Decisions were based on consensus, that is, unanimous agreement among all GDG members. Tables showing the decisions made from evidence to recommendations were developed for the PICO questions.

The full set of tables showing the decisions from evidence to recommendations are included in Online annex 4 (http://www.who.int/tb/areas-of-work/laboratory/annexes_tb_lamp.pdf?ua=1).

2.5. External Review Group

The findings and recommendations from the GDG meeting were sent to an External Review Group of international experts in the field of TB laboratory diagnostics, which included representatives from the WHO TB Supranational Reference Laboratory Network, TB programme managers, and members of the core group of the Global Laboratory Initiative Working Group. The External Review Group did not identify any major errors or missing data in the policy guidance. Members of the External Review Group confirmed that they had no concerns regarding any of the recommendations or any setting-specific issues, nor were there any additional implications for implementation.

3. Scope

This document provides a pragmatic summary of the evidence and recommendations on using TB-LAMP to diagnose pulmonary TB in adults with signs and symptoms consistent with TB. It should be read in conjunction with the WHO's 2015 framework for implementing TB diagnostics, which provides guidance on implementing the diagnostic tools and methods approved by WHO within the context of a country's infrastructure, resources, epidemiology and in the presence of drug-resistant TB and HIV.[9] http://www.who.int/tb/publications/implementing_TB_diagnostics/en/

3.1. Target audience

This guidance is intended to be used by clinicians treating patients with TB, and managers and laboratory directors working in TB programmes, in coordination with external laboratory consultants, donor agencies, technical advisers, laboratory technicians, procurement officers for laboratory equipment, service providers in the private sector, relevant government sectors, and implementation partners that are involved in country-level strengthening of TB diagnostic and treatment services. Individuals responsible for programme planning, budgeting, mobilizing resources and implementing training activities for the programmatic management of drug-resistant TB may also benefit from this document.

[9] Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (WHO/HTM/TB/2015.11; http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf, accessed 22 March 2016).

4. Evidence base for policy formulation

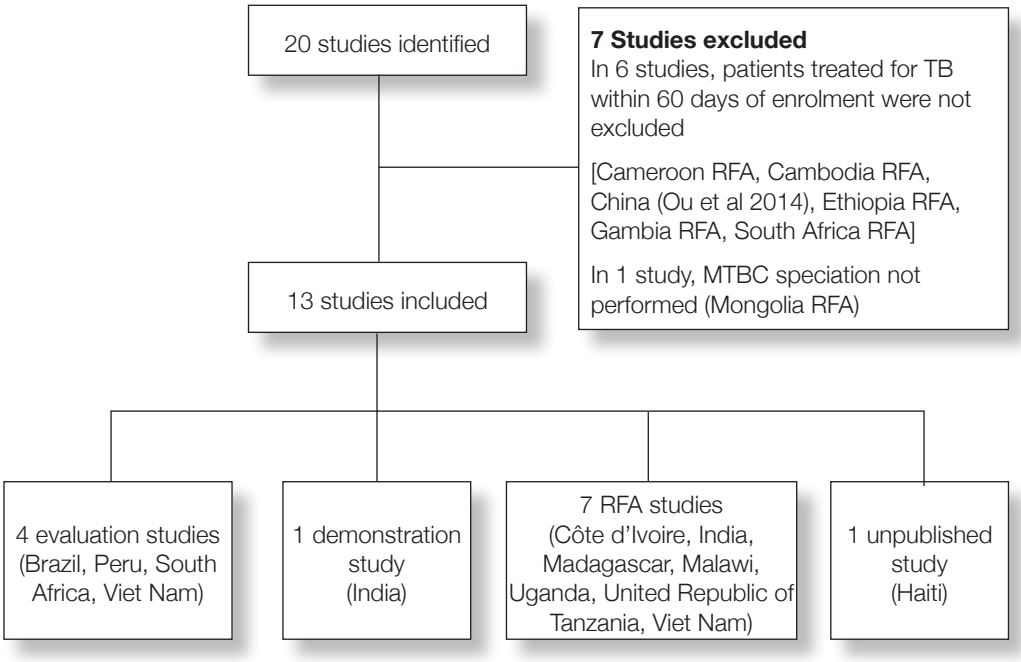
Of the 20 studies identified that evaluated TB-LAMP, 13 studies conducted in 11 countries met the criteria for inclusion in the systematic review (Fig. 4). Six studies were excluded because it was unclear whether participants had been receiving TB treatment within 60 days prior to enrolment. One study was excluded because rapid speciation testing on positive mycobacterial cultures was not performed. (Annex 1 comprises references to the studies identified for the systematic review of the diagnostic accuracy of TB-LAMP.)

Of the 20 studies identified as evaluating TB-LAMP, 10 had not been published (Annex 3 comprises abstracts available for some of the unpublished studies). All 13 included studies provided individual-level data. Of these 13 studies, 5 were conducted by FIND. These were four evaluation studies conducted in reference laboratories in Brazil, Peru and South Africa, and one demonstration study conducted in the setting of intended use, which was performed in rural microscopy centres in India. Seven studies, each performed in a different country using independent protocols, were sponsored by FIND through an RFA. One study in Haiti was conducted without any involvement by FIND, but it was sponsored by the manufacturer, Eiken Chemical Company. The investigators of this study were contacted, and they provided individual-level data.

The 13 studies included in the analysis involved 5 099 participants and were conducted between January 2012 and December 2014. A total of 339 participants were excluded from the analysis. Of these, 22 had a documented history of TB; 1 had had TB-LAMP testing performed on a non-sputum sample; for 111 there were no results from speciation testing for MTBC; 83 were younger than 18 years old; and a further 122 participants had had TB-LAMP testing done with a total reaction volume less than 25 µL. Thus, 4 760 participants across all studies were eligible for inclusion in the analysis.

The characteristics of included studies and the participants enrolled are shown in Table 1. Four studies, all evaluation studies, were conducted at reference laboratories; six studies were conducted at hospital- or university-affiliated clinics; and three were performed at peripheral microscopy centres. All studies performed direct Ziehl–Neelsen or LED fluorescence microscopy. Studies varied in the number and type of media (solid or liquid) used for mycobacterial culture, although all but one study (Madagascar RFA) (Annex 1, Reference 1) performed at least one liquid culture. The liquid culture contamination rate was less than 5% for 7 of 12 studies, including 4 in which the rate was 0%. In addition, all studies performed culture on sputum specimens that had been stored for 1 or more days. Participants in the majority of studies were predominately male, and the median age ranged from 33 years to 60 years. Four of the 13 studies enrolled at least 10% HIV-positive participants (Côte d'Ivoire RFA, Malawi RFA, Uganda RFA, South Africa evaluation). The proportion of patients with culture-positive TB ranged from 20% to 40% for most studies, but was lower in three studies (India demonstration: 11%; India RFA: 15%; Viet Nam RFA: 8%) and higher in one study (Viet Nam evaluation: 66%). The proportion of patients with smear-negative TB varied widely, ranging from 13% (Côte d'Ivoire RFA) to 59% (Viet Nam RFA).

Figure 4. Flow diagram of the 20 TB-LAMP studies identified through a systematic search. (see Annex 1 for information about the included/excluded studies)



MTBC: *Mycobacterium tuberculosis* complex; RFA: request for application

Table 1. Characteristics for all studies included in the evaluation of TB-LAMP (see Annex 1 for information about the studies)

Study	Health system level	Microscopy type	TB culture type	MGIT contamination rate (%)	Type of test done on stored sputum	Xpert MTB/RIF specimen type	Median age (years) of participants (IQR)	Female (%)	HIV-negative[a] (%)	Culture-positive TB[b] (%)	Smear-negative TB[2] (%)
Brazil (EVAL)	Reference Laboratory	2 direct ZN	2 MGIT, 2 LJ	0	Xpert; culture	Frozen processed	48 (35-60)	40	0.4	32	25
Peru (EVAL)	Reference Laboratory	2 direct ZN	2 MGIT, 2 LJ	1.3	Xpert; culture	Fresh processed	43 (28-56)	50	1.0	22	42
S. Africa (EVAL)	Reference Laboratory	2 direct ZN	2 MGIT, 2 LJ	3.1	Xpert; culture	Fresh processed	39 (29-47)	34	35	26	51
Vietnam (EVAL)	Reference Laboratory	2 direct ZN	2 MGIT, 2 LJ	0	Xpert; culture	Frozen processed	39 (26-50)	30	2	66	42
India (DEMO)	Microscopy centre	2 direct ZN	MGIT, LJ	6.5	Culture	None	40 (27-51)	35	2	11	25
India (RFA)	University-affiliated DOTS clinic	1 direct ZN	MGIT	11.5	Xpert; culture	Frozen processed	43 (29-55)	37	2	15	54[3]
Vietnam (RFA)	Microscopy centre	2 direct ZN	MGIT	0	Xpert; culture	Fresh processed	60 (52-70)	42	0.5	8	59
Malawi (RFA)	Microscopy centre	1 direct FM	MGIT, LJ	8.7	Xpert; culture	Fresh direct	35 (26-41)	48	44	16	15[3]
Tanzania (RFA)	District hospital TB clinic	2 direct FM	MGIT, LJ	5.1	Culture	Fresh direct	37 (28-46)	45	6	29	28
Uganda (RFA)	District hospital outpatient clinic	2 direct FM	2 MGIT, 2 LJ	1.3	Culture	Fresh direct	43 (30-54)	43	48	31	45
Ivory Coast (RFA)	District hospital outpatient clinic	2 direct ZN	MGIT	6.0	Xpert Culture	Fresh processed	38 (28-44)	51	12	33	13
Madagascar (RFA)	University-affiliated DOTS clinic	2 direct FM	2 LJ	–	Xpert Culture	Fresh direct	42 (29-52)	40	–	37	27
Haiti	Urban hospital outpatient clinic	2 direct FM	3 MGIT	0	Culture	None	–	–	–	34	23

– Indicates that information was not available.

DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; FM: fluorescence microscopy; IQR: interquartile range; LJ: Löwenstein–Jensen culture; MGIT, mycobacterial growth indicator tube culture (BACTEC MGIT, Becton Dickinson, Franklin Lakes, NJ, United States); RFA: request for application; Xpert: Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, United States); ZN, Ziehl–Neelsen stain.

[a] HIV status was unknown and counted as negative. This reflects the proportion of a study's population that was known to be HIV-positive.

[b] The reference standard used for this calculation was Standard 3 for all studies.

[c] Smear microscopy results were based on the analysis of only one smear.

4.1 Assessment of methodological quality

The quality of the included studies was appraised with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[10] QUADAS-2 consists of four domains: patient selection, index test, reference standard, and flow and timing. All domains were assessed for the potential for risk of bias, and the first three domains for concerns regarding applicability. Signalling questions in each domain were used to form judgements about the risk of bias. Overall, the risk of bias was considered to be high because of problems with the culture-based reference standard (all studies), unclear patient selection (5 of 13 studies), and concerns about flow and timing (8 of 13 studies) (Table 2). Applicability concerns were limited to patient selection in five studies.

Table 2. Risk of bias and applicability concerns: review authors' judgements about each domain (see Annex 1 for information about the studies)

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Brazil (EVAL)	LOW	LOW	HIGH	LOW	HIGH	LOW	LOW
Peru (EVAL)	LOW	LOW	HIGH	LOW	HIGH	LOW	LOW
South Africa (EVAL)	LOW	LOW	HIGH	LOW	HIGH	LOW	LOW
Vietnam (EVAL)	LOW	LOW	HIGH	LOW	HIGH	LOW	LOW
India (DEMO)	LOW	LOW	HIGH	LOW	LOW	LOW	LOW
India (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Vietnam (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Malawi (RFA)	LOW	LOW	HIGH	HIGH	LOW	LOW	LOW
United Republic of Tanzania (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	HIGH	LOW	LOW
Uganda (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Côte d'Ivoire (RFA)	LOW	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Madagascar (RFA)	LOW	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Haiti (unpublished)	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW

DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; RFA: request for applications.

4.1.1 Patient selection

The risk of bias was judged to be unclear for five studies (Haiti unpublished, India RFA, Uganda RFA, United Republic of Tanzania RFA and Viet Nam RFA) because it could not be documented that these studies had excluded patients receiving TB treatment within 60 days of enrolment. Applicability concerns were judged to be high for five studies because they were conducted at reference-level laboratories (Brazil evaluation, Peru evaluation, South Africa evaluation and Viet Nam evaluation) or because enrolment involved patients being screened by a pulmonary specialist (United Republic of Tanzania RFA). All studies enrolled patients consecutively and applied appropriate inclusion and exclusion criteria.

[10] Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011; 155(8):529–36. doi: 10.7326/0003-4819-155-8-201110180-00009.

4.1.2 Index test (TB-LAMP)

The risk of bias was judged to be low for all studies because TB-LAMP testing was performed in accordance with the latest protocol, and TB-LAMP operators were blinded to the results of other tests. All studies used standard reporting of the results of the initial TB-LAMP test as negative, positive, or indeterminate or invalid.

4.1.3 Reference standard

The risk of bias was judged to be high for all studies because of the failure to perform mycobacterial culture on at least two sputum samples (Côte d'Ivoire RFA, India demonstration, India RFA, Malawi RFA, United Republic of Tanzania RFA, Viet Nam RFA), failure to use liquid culture (Madagascar RFA) or because liquid culture contamination rates were outside the acceptable range of 5–12% (Brazil evaluation, Peru evaluation, South Africa evaluation, Viet Nam evaluation, India RFA, Uganda RFA, Viet Nam RFA and Haiti unpublished). There were judged to be low concerns about applicability for all studies because TB culture is a recognized reference method for the bacteriological confirmation of TB.

4.1.4 Flow and timing

The risk of bias was judged to be high for one study (Malawi RFA) because more than 20% of eligible participants were excluded from the analysis using the most stringent culture-based reference standard for which the study qualified. The risk of bias was judged to be unclear for seven studies (Côte d'Ivoire RFA, India RFA, Madagascar RFA, Uganda RFA, United Republic of Tanzania RFA, Viet Nam RFA and Haiti unpublished) due to a lack of information regarding the percentage of eligible participants at each site who had data that were ultimately submitted for this analysis.

5. Results of the systematic review

5.1 Accuracy of TB-LAMP as a replacement test for smear microscopy

Of the 4 760 adults eligible for inclusion in the analysis, 1 810 participants (38%) across 7 studies qualified for Standard 1 status; 3 110 participants (65%) across 10 studies qualified for Standard 2; and 4 596 participants (97%) across 13 qualified for Standard 3 (Table 3).

Table 3. TB-LAMP as a replacement test for smear microscopy: eligible and included patients, by reference standard and study site (see Annex 1 for information about the studies)

Study	Total number of participants	Number of participants eligible[a]	Number (%) included[b]		
			Standard 1	Standard 2	Standard 3
Brazil (EVAL)	266	239	237 (99)	237 (99)	237 (99)
Peru (EVAL)	199	198	198 (100)	198 (100)	198 (100)
South Africa (EVAL)	259	240	237 (99)	237 (99)	238 (99)
Vietnam (EVAL)	312	304	304 (100)	304 (100)	304 (100)
India (DEMO)	619	598	–	559 (94)	586 (98)
India (RFA)	530	504	–	–	446 (89)
Vietnam (RFA)	503	364	–	–	361 (99)
Malawi (RFA)	273	265	–	149 (56)	234 (88)
Unite Republic of Tanzania (RFA)	648	648	–	587 (91)	632 (98)
Uganda (RFA)	233	190	184 (97)	189 (99)	190 (100)
Côte d'Ivoire (RFA)	500	480	–	–	451 (94)
Madagascar (RFA)	548	521	476 (91)	476 (91)	516 (99)
Haiti (unpublished)	209	209	174 (83)	174 (83)	203 (97)
Total	**5 099**	**4 760**	**1 810 (38)**	**3 110 (65)**	**4 596 (97)**

– Indicates that reference standard criteria were not met by at least 5 TB patients and 5 non-TB patients.
DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; RFA: request for application.

[a] The total number eligible includes those with missing or indeterminate TB-LAMP results: Brazil EVAL (n = 2 indeterminate), South Africa EVAL (n = 2 indeterminate), Madagascar RFA (n = 1 missing, n = 1 indeterminate), Malawi RFA (n = 5 missing), United Republic of Tanzania RFA (n = 11 missing), Haiti unpublished (n = 6 missing).

[b] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients had to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).

For Standard 1, the sensitivity of TB-LAMP in individual studies ranged from 66% to 82%; for Standard 2, it ranged from 62% to 91%; and for Standard 3, it ranged from 48% to 100% (Fig. 5). There was significant heterogeneity in the sensitivity estimates, both from visual inspection of forest plots and statistical testing (for all reference standards, I2: 72–94%; $P < 0.003$). The pooled sensitivity for TB-LAMP was higher than for smear microscopy, ranging from 77.7% (95% confidence interval [CI]: 71.2–83.0) to 80.3% (95% CI: 70.3–87.5) (Table 4). When sensitivity differences were pooled across studies, TB-LAMP ranged from being 7.1% (95% CI: 1.4–12.9) to 13.2% (95% CI: 4.5–21.9) more sensitive than sputum-smear microscopy, depending on the reference standard used. The

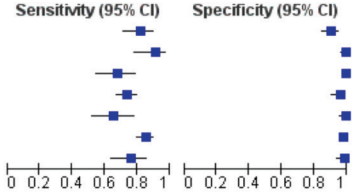
pooled sensitivity for TB-LAMP among sputum smear-positive patients ranged from 95.2% (95% CI: 90.2–97.7) to 96.6% (91.9–98.6) across studies, depending on the reference standard used.

For Standard 1, the specificity of TB-LAMP in individual studies ranged from 90% to 99%; for Standards 2 and 3, it was 90–100% (Fig. 5). Visual inspection of forest plots indicated that the heterogeneity in specificity estimates was less than that for sensitivity estimates, but it was still significant (for all reference standards, I2: 61–78%; $P < 0.03$). The pooled specificity of TB-LAMP ranged from 97.7% (95% CI: 96.1–98.7) for Standard 3 to 98.1% (95% CI: 95.7–99.2) for Standard 1 (Table 4). When specificity differences were pooled across studies, TB-LAMP performed similarly to sputum-smear microscopy (pooled specificity differences ranged from -1.8% [95% CI: -3.8 to +0.2] to -1.3% [95% CI: -3.1 to +0.4]), depending on the reference standard used.

Figure 5. Forest plots of TB-LAMP sensitivity and specificity for detecting TB compared with three culture-based reference standards[a] (See Annex 1 for information about the studies)

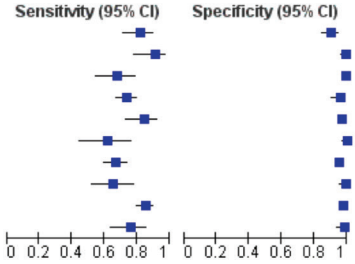
Standard 1

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	62	16	14	145	0.82 [0.71, 0.90]	0.90 [0.84, 0.94]
Peru (Eval)	39	1	4	154	0.91 [0.78, 0.97]	0.99 [0.96, 1.00]
South Africa (Eval)	42	1	20	174	0.68 [0.55, 0.79]	0.99 [0.97, 1.00]
Vietnam (Eval)	149	4	53	98	0.74 [0.67, 0.80]	0.96 [0.90, 0.99]
Uganda (RFA)	38	1	20	125	0.66 [0.52, 0.78]	0.99 [0.96, 1.00]
Madagascar (RFA)	161	6	28	281	0.85 [0.79, 0.90]	0.98 [0.96, 0.99]
Haiti (Unpublished)	50	2	16	106	0.76 [0.64, 0.85]	0.98 [0.93, 1.00]



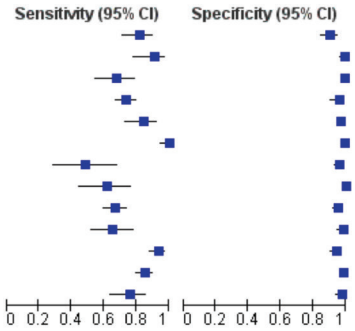
Standard 2

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	62	16	14	145	0.82 [0.71, 0.90]	0.90 [0.84, 0.94]
Peru (Eval)	39	1	4	154	0.91 [0.78, 0.97]	0.99 [0.96, 1.00]
South Africa (Eval)	42	1	20	174	0.68 [0.55, 0.79]	0.99 [0.97, 1.00]
Vietnam (Eval)	149	4	53	98	0.74 [0.67, 0.80]	0.96 [0.90, 0.99]
India (Demo)	53	15	10	481	0.84 [0.73, 0.92]	0.97 [0.95, 0.98]
Malawi (RFA)	24	0	15	110	0.62 [0.45, 0.77]	1.00 [0.97, 1.00]
Tanzania (RFA)	122	19	60	386	0.67 [0.60, 0.74]	0.95 [0.93, 0.97]
Uganda (RFA)	38	1	20	130	0.66 [0.52, 0.78]	0.99 [0.96, 1.00]
Madagascar (RFA)	161	6	28	281	0.85 [0.79, 0.90]	0.98 [0.96, 0.99]
Haiti (Unpublished)	50	2	16	106	0.76 [0.64, 0.85]	0.98 [0.93, 1.00]



Standard 3

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	62	16	14	145	0.82 [0.71, 0.90]	0.90 [0.84, 0.94]
Peru (Eval)	39	1	4	154	0.91 [0.78, 0.97]	0.99 [0.96, 1.00]
South Africa (Eval)	42	1	20	175	0.68 [0.55, 0.79]	0.99 [0.97, 1.00]
Vietnam (Eval)	149	4	53	98	0.74 [0.67, 0.80]	0.96 [0.90, 0.99]
India (Demo)	53	15	10	508	0.84 [0.73, 0.92]	0.97 [0.95, 0.98]
India (RFA)	65	3	0	378	1.00 [0.94, 1.00]	0.99 [0.98, 1.00]
Vietnam (RFA)	13	14	14	320	0.48 [0.29, 0.68]	0.96 [0.93, 0.98]
Malawi (RFA)	24	0	15	195	0.62 [0.45, 0.77]	1.00 [0.98, 1.00]
Tanzania (RFA)	122	23	60	427	0.67 [0.60, 0.74]	0.95 [0.92, 0.97]
Uganda (RFA)	38	2	20	130	0.66 [0.52, 0.78]	0.98 [0.95, 1.00]
Ivory Coast (RFA)	140	18	10	283	0.93 [0.88, 0.97]	0.94 [0.91, 0.96]
Madagascar (RFA)	161	6	28	321	0.85 [0.79, 0.90]	0.98 [0.96, 0.99]
Haiti (Unpublished)	50	3	16	134	0.76 [0.64, 0.85]	0.98 [0.94, 1.00]



CI: confidence interval; DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; FN: false negative; FP: false positive; RFA: request for application; TN: true negative; TP: true positive.
[a] The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Table 4. TB-LAMP as a replacement test for smear microscopy: estimates of pooled sensitivity and specificity

Reference standard[a]	Pooled sensitivity[b]	Pooled specificity[b]
Standard 1	77.7 (71.2-83.0)	98.1 (95.7-99.2)
Standard 2	76.0 (69.9-81.2)	98.0 (96.0-99.0)
Standard 3	80.3 (70.3-87.5)	97.7 (96.1-98.7)

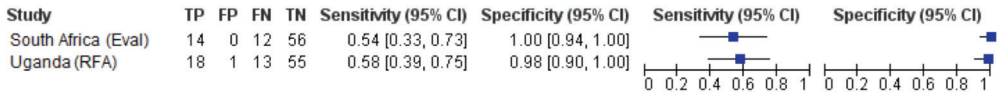
[a] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients had to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).
[b] Values are percentages (95% confidence intervals).

5.2. Accuracy of TB-LAMP as a replacement test for smear microscopy in persons living with HIV

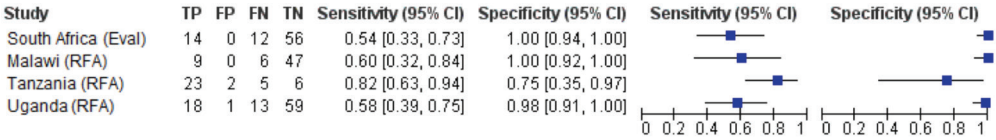
Most studies did not collect data on HIV status and most patients had unknown HIV status at the time of enrolment. Of the 385 adults with known HIV infection who were eligible for inclusion in the analysis, 169 participants (44%) in 2 studies qualified for Standard 1 status; 271 (70%) across 4 studies qualified for Standard 2; and 370 (96%) across 5 studies qualified for Standard 3. For Standard 1, the sensitivity of TB-LAMP in individual studies ranged from 54% to 58%; for Standard 2, the range was 54–82%; and for Standard 3, the range was 54–96% (Fig. 6). The corresponding ranges for TB-LAMP specificity in individual studies were, respectively, 98–100%, 75–100% and 33–100%.

Figure 6: Forest plots of TB-LAMP sensitivity and specificity for detecting TB among adults living with HIV compared with three culture-based reference standards [a] ***(See Annex 1 for information about the studies)***

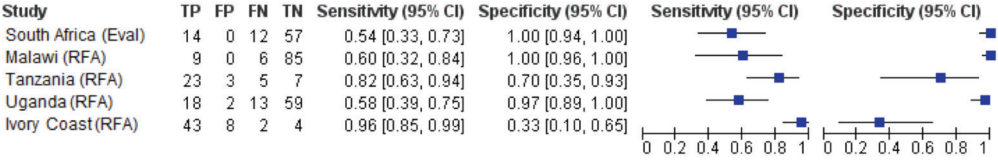
Standard 1



Standard 2



Standard 3



CI confidence interval; EVAL: evaluation study conducted in a reference laboratory; FN: false negative; FP: false positive; RFA: request for application; TN: true negative; TP: true positive.
[a] The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Visual inspection of forest plots indicated there was more heterogeneity in sensitivity estimates and specificity estimates when using Standard 3 than when using Standard 2. (There were insufficient studies to evaluate these for Standard 1.) For Standard 2, heterogeneity was moderate but not significant for sensitivity (I^2: 54%; P = 0.09) and negligible for specificity (I^2: 0%; $P = 0.42$). For Standard 3, heterogeneity was significant for both sensitivity (I^2: 86%; $P < 0.001$) and specificity (I^2: 85%; $P < 0.001$).

The pooled sensitivity of TB-LAMP among HIV-positive adults was lower than among all adults, ranging from 63.8% (95% CI: 49.0–76.4) for Standard 2 to 73.4% (95% CI: 51.9–87.6) for Standard 3 (Table 5). Pooled specificity was low for Standard 3 (95.0%; 95% CI: 64.0–99.5) but high for Standard 2 (98.8%; 95% CI: 85.1–99.9).

Table 5. TB-LAMP as a replacement test for smear microscopy in persons living with HIV: pooled sensitivity and specificity

Reference standard[a]	Pooled sensitivity[b]	Pooled specificity[b]
Standard 1	NA	NA
Standard 2	63.8 (49.0-76.4)	98.8 (85.1-99.9)
Standard 3	73.4 (51.9-87.6)	95.0 (64.0-99.5)

NA: not applicable because < 4 studies were eligible.
[a] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).
[b] Values are percentages (95% confidence intervals).

5.3. Accuracy of TB-LAMP as an add-on test following smear microscopy for smear-negative adults

The diagnostic accuracy of TB-LAMP for detecting smear-negative culture-confirmed pulmonary TB was limited to adult participants with two negative sputum-smear microscopy results. Of 2 972 participants eligible for the analysis, 1 349 (45%) across 7 studies qualified for Standard 1 status; 2 190 (74%) across 9 studies qualified for Standard 2; and 2 916 (98%) across 11 studies qualified for Standard 3 (Table 6).

Table 6. TB-LAMP as an add-on test following negative sputum-smear microscopy: eligible and included patients, by reference standard and study site (see Annex 1 for information about the studies)

Study	Total number of participants	Number of participants eligible[a]	Number (%) included[b]		
			Standard 1	Standard 2	Standard 3
Brazil (EVAL)	266	182	180 (99)	180 (99)	180 (99)
Peru (EVAL)	199	173	173 (100)	173 (100)	173 (100)
South Africa (EVAL)	259	207	204 (99)	204 (99)	205 (99)
Vietnam (EVAL)	312	186	186 (100)	186 (100)	186 (100)
India (DEMO)	619	432	–	403 (93)	421 (98)
India (RFA)	530	0	–	–	–
Vietnam (RFA)	503	351	–	–	348 (99)
Malawi (RFA)	273	0	–	–	–
United Republic of Tanzania (RFA)	648	489	–	438 (90)	478 (98)
Uganda (RFA)	233	149	149 (100)	149 (100)	149 (100)
Côte d'Ivoire (RFA)	500	329	–	–	305 (93)
Madagascar (RFA)	548	350	333 (95)	333 (95)	347 (99)
Haiti (Unpublished)	209	124	124 (100)	124 (100)	124 (100)
Total	**5 099**	**2 972**	**1 349 (45)**	**2 190 (74)**	**2 916 (98)**

– Indicates that reference standard criteria were not met by at least 5 TB patients and 5 non-TB patients.
DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; RFA: request for application.
[a] The total number eligible includes those with missing or indeterminate TB-LAMP results: Brazil EVAL (*n* = 2 indeterminate), South Africa EVAL (*n* = 2 indeterminate), Madagascar RFA (*n* = 1 indeterminate), United Republic of Tanzania RFA (*n* = 6 missing).
[b] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).

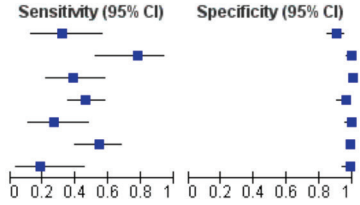
For Standard 1, the sensitivity of TB-LAMP in individual studies ranged from 19% to 78%; for Standard 2, it ranged from 19% to 81% ; and for Standard 3, it ranged from 17% to 81% (Fig. 7). There was significant heterogeneity in sensitivity estimates across studies, both from visual inspection of forest plots and statistical testing (for all reference standards, I2: 74–86%; $P < 0.001$). As expected, the pooled sensitivity of TB-LAMP was lower among smear-negative culture-positive adults than among all adults, ranging from 40.3% (95% CI: 27.9–54.0) to 42.2% (95% CI: 27.9–57.9) (Table 7).

For all standards, the specificity of TB-LAMP in individual studies ranged from 90% to 100% (Fig. 7). Visual inspection of forest plots indicated that heterogeneity in specificity estimates was less than that for sensitivity estimates across studies, but it was still significant (for all reference standards, I^2: 67–70.3%; $P < 0.005$). The pooled specificity of TB-LAMP among smear-negative culture-positive adults was similar to that observed among all adults, ranging from 97.7% (95% CI: 96.1–98.6) for Standard 3 to 98.4% (95% CI: 95.9–99.4) for Standard 1 (Table 7).

Figure 7. Forest plots of TB-LAMP sensitivity and specificity for detecting TB among adults as an add-on test following negative sputum-smear microscopy compared with three culture-based reference standards [a] (See Annex 1 for information about the studies)

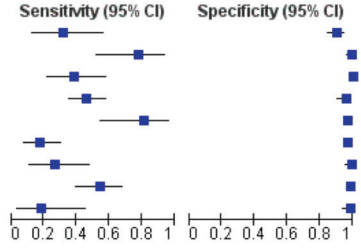
Standard 1

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	6	16	13	145	0.32 [0.13, 0.57]	0.90 [0.84, 0.94]
Peru (Eval)	14	1	4	154	0.78 [0.52, 0.94]	0.99 [0.96, 1.00]
South Africa (Eval)	12	0	19	173	0.39 [0.22, 0.58]	1.00 [0.98, 1.00]
Vietnam (Eval)	39	4	45	98	0.46 [0.35, 0.58]	0.96 [0.90, 0.99]
Uganda (RFA)	7	1	19	122	0.27 [0.12, 0.48]	0.99 [0.96, 1.00]
Madagascar (RFA)	27	4	23	279	0.54 [0.39, 0.68]	0.99 [0.96, 1.00]
Haiti (Unpublished)	3	2	13	106	0.19 [0.04, 0.46]	0.98 [0.93, 1.00]



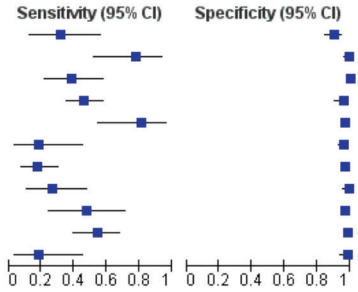
Standard 2

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	6	16	13	145	0.32 [0.13, 0.57]	0.90 [0.84, 0.94]
Peru (Eval)	14	1	4	154	0.78 [0.52, 0.94]	0.99 [0.96, 1.00]
South Africa (Eval)	12	0	19	173	0.39 [0.22, 0.58]	1.00 [0.98, 1.00]
Vietnam (Eval)	39	4	45	98	0.46 [0.35, 0.58]	0.96 [0.90, 0.99]
India (Demo)	13	12	3	375	0.81 [0.54, 0.96]	0.97 [0.95, 0.98]
Tanzania (RFA)	9	12	43	374	0.17 [0.08, 0.30]	0.97 [0.95, 0.98]
Uganda (RFA)	7	1	19	122	0.27 [0.12, 0.48]	0.99 [0.96, 1.00]
Madagascar (RFA)	27	4	23	279	0.54 [0.39, 0.68]	0.99 [0.96, 1.00]
Haiti (Unpublished)	3	2	13	106	0.19 [0.04, 0.46]	0.98 [0.93, 1.00]



Standard 3

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Brazil (Eval)	6	16	13	145	0.32 [0.13, 0.57]	0.90 [0.84, 0.94]
Peru (Eval)	14	1	4	154	0.78 [0.52, 0.94]	0.99 [0.96, 1.00]
South Africa (Eval)	12	0	19	174	0.39 [0.22, 0.58]	1.00 [0.98, 1.00]
Vietnam (Eval)	39	4	45	98	0.46 [0.35, 0.58]	0.96 [0.90, 0.99]
India (Demo)	13	12	3	393	0.81 [0.54, 0.96]	0.97 [0.95, 0.98]
Vietnam (RFA)	3	14	13	318	0.19 [0.04, 0.46]	0.96 [0.93, 0.98]
Tanzania (RFA)	9	13	43	413	0.17 [0.08, 0.30]	0.97 [0.95, 0.98]
Uganda (RFA)	7	1	19	122	0.27 [0.12, 0.48]	0.99 [0.96, 1.00]
Ivory Coast (RFA)	9	8	10	278	0.47 [0.24, 0.71]	0.97 [0.95, 0.99]
Madagascar (RFA)	27	4	23	293	0.54 [0.39, 0.68]	0.99 [0.97, 1.00]
Haiti (Unpublished)	3	2	13	106	0.19 [0.04, 0.46]	0.98 [0.93, 1.00]



CI confidence interval; DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; FN: false negative; FP: false positive; RFA: request for application; TN: true negative; TP: true positive.
[a] The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). Values for test results are the number of each type of result (true positive, false positive, false negative, true negative).

Table 7. TB-LAMP as an add-on test following smear microscopy: pooled sensitivity and specificity

Reference standard[a]	Pooled sensitivity[b]	Pooled specificity[b]
Standard 1	42.1 (30.0-55.3)	98.4 (95.9-99.4)
Standard 2	42.2 (27.9-57.9)	98.0 (96.0-99.0)
Standard 3	40.3 (27.9-54.0)	97.7 (96.1-98.6)

[a] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).
[b] Values are percentages (95% confidence intervals).

5.4 Accuracy of TB-LAMP compared with the Xpert MTB/RIF assay for detecting pulmonary TB

The difference in diagnostic accuracy between TB-LAMP and the Xpert MTB/RIF assay for detecting pulmonary TB compared with culture as the reference standard was determined for participants who underwent both TB-LAMP and Xpert MTB/RIF testing on non-frozen specimens. Of 2 837 participants eligible for inclusion in the analysis, 1 075 (38%) across four studies qualified for Standard 1 status; 1 809 (64%) across 6 studies qualified for Standard 2; and 2 772 (98%) across eight studies qualified for Standard 3 (Table 8).

Table 8. Diagnostic accuracy of TB-LAMP compared with the Xpert MTB/RIF assay: eligible and included patients, by reference standard and study site. (See Annex 1 for information about the studies)

Study	Total number of participants	Number of participants eligible[a]	Number (%) included[b]		
			Standard 1	Standard 2	Standard 3
Brazil (EVAL)	266	–	–	–	–
Peru (EVAL)	199	190	190 (100%)	190 (100%)	190 (100%)
South Africa (EVAL)	259	238	237 (99%)	237 (99%)	238 (100%)
Vietnam (EVAL)	312	–	–	–	–
India (DEMO)	619	–	–	–	–
India (RFA)	530	–	–	–	–
Vietnam (RFA)	503	344	–	–	342 (99%)
Malawi (RFA)	273	258	–	148 (57%)	232 (90%)
United Republic of Tanzania (RFA)	648	630	–	581 (92%)	625 (99%)
Uganda (RFA)	233	190	184 (97%)	189 (99%)	190 (100%)
Ivory Coast (RFA)	500	480	–	–	451 (94%)
Madagascar (RFA)	548	507	464 (92%)	464 (92%)	504 (99%)
Haiti (Unpublished)	209	–	–	–	–
Total	**5 099**	**2 837**	**1 075 (38%)**	**1 809 (64%)**	**2 772 (98%)**

– Indicates that reference standard criteria were not met by at least 5 TB patients and 5 non-TB patients.
DEMO: demonstration study conducted in the setting of intended use; EVAL: evaluation study conducted in a reference laboratory; RFA: request for application.

[a] The total number eligible includes only those patients with positive or negative TB-LAMP and Xpert MTB/RIF results.

[b] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).

In this analysis, the pooled sensitivity of TB-LAMP ranged from 74.1% (95% CI: 64.1–82.2) to 78.0% (95% CI: 66.6–86.4) between standards, and the pooled specificity ranged from 98.2% (95% CI: 96.0–99.2) to 98.9% (95% CI: 97.4–99.6) across reference standards (Table 9). The pooled sensitivity of the Xpert MTB/RIF assay ranged from 80.4% (95% CI: 73.4–85.9) to 84.0% (95% CI: 75.6–90.0), and the pooled specificity ranged from 97.2% (95% CI: 94.4–98.6) to 98.2% (95% CI: 95.9–99.2) across reference standards.

Table 9. Accuracy of TB-LAMP and the Xpert MTB/RIF assay: pooled sensitivity and specificity

Reference standard[a]	Pooled sensitivity[c]	Pooled specificity[c]
TB-LAMP[b]		
Standard 1	78.0 (66.6-86.4)	98.9 (97.4-99.6)
Standard 2	74.1 (64.1-82.2)	98.8 (96.8-99.6)
Standard 3	75.8 (63.2-85.0)	98.2 (96.0-99.2)
Xpert MTB/RIF[b]		
Standard 1[2]	81.1 (70.6-88.5)	98.2 (95.9-99.2)
Standard 2[2]	80.4 (73.4-85.9)	97.4 (94.9-98.7)
Standard 3[2]	84.0 (75.6-90.0)	97.2 (94.4-98.6)

[a] Data were restricted to study participants for whom there were valid results for both TB-LAMP and the Xpert MTB/RIF assay and cases in which testing was performed on non-frozen specimens.
[b] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).
[c] Values are percentages (95% confidence intervals).

5.5 Evaluation of the proportion of indeterminate or invalid results when TB-LAMP is used to detect pulmonary TB among all adults and among HIV-positive adults

All 4 760 patients eligible for the analysis of the accuracy of TB-LAMP among adults were included in the analysis to determine the proportion of indeterminate or invalid TB-LAMP results (Table 3). The proportion of indeterminate TB-LAMP results was 0% in 11 studies and 1% in 2 studies. There was minimal heterogeneity across studies (I^2: 28%; P = 0.25). The pooled proportion of indeterminate TB-LAMP results was 0% (95% CI: 0–0). Results were similar among HIV-positive adults; the pooled proportion of indeterminate TB-LAMP results in this subgroup was 0% (95% CI: 0–1).

6. Cost-effectiveness analysis

This analysis provides evidence for the cost–effectiveness of TB-LAMP as a replacement test for sputum-smear microscopy or as an add-on test to sputum-smear microscopy in the further testing of smear-negative patients compared with the standard of care in settings where coverage of the Xpert MTB/RIF assay is limited (H. Sohn, Cost, affordability, and cost-effectiveness of TB-LAMP assay: report to WHO Guideline Development Group meeting on the TB-LAMP assay, unpublished report, January 2016).

For the cost analysis, a bottom-up micro-costing analysis was conducted, aiming to identify, measure and value all resources relevant to providing TB-LAMP and the Xpert MTB/RIF assay as routine diagnostic tests in peripheral laboratories in Malawi and Viet Nam. Affordability was assessed using the national budgets for TB control reported to WHO for Malawi and Viet Nam as a reference for the total expected implementation cost of a complete roll-out of the two technologies at peripheral-level microscopy laboratories. The two TB-LAMP strategies (use as a replacement test for sputum-smear microscopy and as an add-on test to sputum-smear microscopy for further testing in smear-negative patients) were compared with the base case algorithm, with sputum-smear microscopy followed by clinical diagnosis in those patients with a negative microscopy result.

The weighted average per-test cost of TB-LAMP was between US$ 13.78 and US$ 16.22 and for the Xpert MTB/RIF assay it was US$ 19.17 to US$ 28.34 when they were used as routine diagnostic tests at all peripheral-level laboratories in both countries. The first-year expenditure required for implementation at peripheral laboratories with a medium workload (10–15 sputum-smear microscopy tests per day) in Viet Nam was US$ 26 917 for TB-LAMP and US$ 43 325 for the Xpert MTB/RIF assay. These costs were approximately US$ 3 000 lower in Malawi, attributable to lower operating and staff costs. Likewise, TB-LAMP was a considerably cheaper test to implement, accounting for 9.33% in Malawi and 17.2% in Viet Nam of the reported TB control budget for 2014 compared with implementing the Xpert MTB/RIF assay, which accounted for 18% in Malawi and 37% in Viet Nam. In the cost–effectiveness analyses, both of the TB-LAMP scenarios improved case-detection rates and both strategies were cost–effective when compared with WHO's willingness-to-pay threshold levels.

As a test performed at peripheral laboratories, TB-LAMP is generally a cheaper and more affordable molecular test alternative to the Xpert MTB/RIF assay. The findings of the cost–effectiveness analysis demonstrate that TB-LAMP is potentially a cost–effective alternative to the base case of sputum-smear microscopy plus clinical diagnosis in settings where the Xpert MTB/RIF assay cannot be implemented due to its infrastructure requirements, including a continuous power supply. However, given the inability of TB-LAMP to detect rifampicin-resistant TB, and its suboptimal sensitivity for detecting TB among persons living with HIV, policy-makers must cautiously evaluate the operational feasibility and cost considerations prior to introducing this technology to their countries.

7. Summary: from evidence to recommendations

The accuracy of TB-LAMP performed directly on sputum samples for diagnosing pulmonary TB among adults who have not received TB treatment within 60 days of enrolment was determined from 13 studies performed in countries with intermediate and high burdens of TB.

7.1. TB-LAMP for detecting pulmonary TB in all adults

Using data from the 1 810 persons with signs and symptoms consistent with TB for whom the most stringent reference standard could be used (Standard 1), TB-LAMP had a pooled sensitivity that was 15% higher than smear microscopy (78% compared with 63%). Although pooled specificity estimates for TB-LAMP were 2% lower (98% compared with 100%), this may be partly explained by the identification of TB cases who were misclassified as TB-negative by the use of culture as a reference standard, which varied across studies. All included studies were considered to have a high risk of bias in the use of culture as a reference standard, leading to the misclassification of patients.

The GDG felt that the anticipated possible desirable effect was the diagnosis of additional TB-positive cases that would be missed by the use of smear microscopy alone. TB-LAMP would correctly identify 7 more cases per 1 000 individuals tested if the pre-test probability of TB is 5%, and TB-LAMP would identify 22 more cases per 1 000 individuals tested if the pre-test probability of TB is 15% (Annex 2, Table 10). The correct identification of additional TB cases would be expected to lead to higher cure rates, fewer sequelae for the patient, and less transmission in the community.

The anticipated undesirable effect is the incorrect identification of individuals as TB cases when they are actually TB-negative (a false positive). In this pooled data, TB-LAMP had inferior performance to smear microscopy, leading to an estimate of 16 more cases misclassified per 1 000 individuals tested if the pre-test probability of TB is 5%; it would lead to 14 more cases misclassified per 1 000 individuals tested if the pre-test probability of TB is 15% (Annex 2, Table 10). Incorrectly identifying an individual as TB-positive would lead to inappropriate treatment with potential medication toxicities for the individual, the possible negative effect of stigmatization for the individual, and negative economic effects for the individual and society. With a better reference standard, it would be expected that some false-positive TB-LAMP results would be reclassified as true positives, leading to improved sensitivity and specificity. Patients with non-tuberculous mycobacteria were excluded from this analysis, but will be present in programmatic settings, being detected as false-positive results by smear microscopy, which would decrease the specificity of sputum-smear microscopy. Thus, the GDG felt that it would be expected that using TB-LAMP as a replacement for sputum-smear microscopy would lead to more TB cases being identified while keeping false-positive results to an acceptable minimum (see Online annex 4).

7.2. TB-LAMP for detecting pulmonary TB in adults living with HIV

Data from 4 studies (271 participants) that included HIV-positive adults with signs and symptoms consistent with pulmonary TB and that evaluated the accuracy of TB-LAMP demonstrated sensitivity and specificity similar to sputum-smear microscopy (sensitivity: 64% for TB-LAMP and 62% for smear microscopy; specificity: 99% for TB-LAMP and 99% for smear microscopy)(Annex 2, Table 11). Based on this limited dataset, similar numbers of true-positive, false-negative, false-positive and true-negative results would be obtained using either TB-LAMP or sputum-smear microscopy. Although it would be expected that TB-LAMP would have a higher sensitivity than smear microscopy in HIV-positive adults, given the 42% incremental yield observed in detecting TB using TB-LAMP in all patients with culture-confirmed TB and negative sputum-smear microscopy (see Section 7.3), this was not evident in the data from the 271 HIV-positive patients evaluated for this review.

The GDG felt that these findings suggest that TB-LAMP is less sensitive among HIV-positive adults than among all adults suspected of having TB, likely due to a higher proportion of patients with sputum smear-negative TB in this population. As a consequence, the GDG members decided not to make a specific recommendation for or against using TB-LAMP in HIV-positive persons but to reflect that there was limited evidence (Online annex 4).

7.3. TB-LAMP as an add-on test for detecting of pulmonary TB in adults following negative smear microscopy

Using data from the 1 349 persons with signs and symptoms consistent with TB in the 7 studies to which the most stringent reference standard could be applied (Standard 1), TB-LAMP showed a 42% incremental yield in patients with culture-confirmed TB who had a negative sputum-smear microscopy result. Pooled specificity estimates for TB-LAMP were 2% lower (98% versus 100% for smear microscopy), and this may be partly explained by the identification of TB cases that were misclassified as TB-negative using culture as a reference standard, which varied across studies. All included studies were considered to have a high risk of bias in the use of culture as a reference standard, which would lead to the misclassification of patients.

The GDG felt that the anticipated desirable effect was the diagnosis of additional TB-positive cases that would be missed by the use of smear microscopy alone. TB-LAMP would correctly identify 21 more cases per 1 000 individuals tested if the pre-test probability of TB is 5%; it would identify 63 more cases per 1 000 individuals tested if the pre-test probability of TB is 15% (Annex 2, Table 12). The correct identification of additional TB cases would be expected to lead to higher cure rates, fewer sequelae for the patient, and less transmission in the community.

The anticipated undesirable effect is the incorrect identification of individuals as TB cases when they are actually TB negative (false positive). In the pooled data, TB-LAMP had inferior performance to smear microscopy, leading to an estimate of 19 more cases misclassified per 1 000 individuals tested if the pre-test probability of TB is 5%; 17 more cases would be misclassified per 1 000 individuals tested if the pre-test probability of TB is 15% (Annex 2, Table 12). The incorrect identification of an individual as TB-positive would lead to inappropriate treatment with potential medication toxicities for the individual, the possible negative effects of stigmatization for the individual, and negative economic effects for the individual and society. With a better reference standard, it could be expected that some false-positive TB-LAMP results would be reclassified as true positives, leading to improved sensitivity and specificity. Thus the GDG felt that using TB-LAMP as an add-on test following negative sputum-smear microscopy would lead to more TB cases being identified while keeping false-positive results to an acceptable minimum (Online annex 4).

7.4 TB-LAMP as a replacement test for the Xpert MTB/RIF assay for detecting pulmonary TB in adults

Although better than microscopy, the pooled sensitivity of TB-LAMP is lower than what has been reported for the Xpert MTB/RIF assay (89%; 95% credible interval: 85–92).[11] The specificity of all three tests is similar. In head-to-head comparisons, TB-LAMP appeared to be less sensitive than the Xpert MTB/RIF assay, but the difference in sensitivity was not statistically significant except when using the least stringent reference standard. More data are needed to confirm that TB-LAMP sensitivity is lower than that of the Xpert MTB/RIF assay. It is also important to consider that TB-LAMP does not detect rifampicin resistance.

[11] Steingart KR, Schiller I, Horne D, Pai M, Boehme CC, Dendukuri N. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev.. 2014;(1):CD009593. doi:10.1002/14651858.CD009593.pub3.

The GDG felt that TB-LAMP could be implemented as a replacement test for microscopy in peripheral microscopy centres where the laboratory infrastructure and requirements for continuous power supply restrict the implementation of the Xpert MTB/RIF assay. The evidence along with the increased automation of, the fewer training requirements for, and the ability to identify rifampicin resistance suggest that the Xpert MTB/RIF assay should remain the preferred diagnostic wherever there are sufficient resources and infrastructure to support its use. The GDG acknowledged the importance of reaching the targets in the End TB Strategy that prioritize the implementation of diagnostics that allow for the early diagnosis of TB, including universal access to drug-susceptibility testing. As such, the GDG decided not to develop an evidence-to-recommendation table for this PICO question.

8. WHO policy recommendations

Given the GRADE evidence assessment and considering the relative benefits and harms associated with the use of the TB-LAMP, WHO recommends the following.

1. TB-LAMP may be used as a replacement test for sputum-smear microscopy to diagnose pulmonary TB in adults with signs and symptoms consistent with TB (conditional recommendation, very low-quality evidence).
2. TB-LAMP may be used as a follow-on test to smear microscopy in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary (conditional recommendation, very low-quality evidence).

Remarks

- These recommendations apply to settings where conventional sputum-smear microscopy can be performed.
- TB-LAMP should not replace the use of rapid molecular tests that detect TB and resistance to rifampicin, especially among populations at risk of multidrug-resistant TB.
- Due to the limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over sputum-smear microscopy for testing persons living with HIV who have signs and symptoms consistent with TB.
- These recommendations apply only to the use of TB-LAMP in testing sputum specimens from patients with signs and symptoms consistent with pulmonary TB.
- These recommendations are extrapolated to using TB-LAMP in children, based on the generalization of data from adults, while acknowledging the difficulties of collecting sputum specimens from children.

9. Implementation considerations

The systematic review supports the use of TB-LAMP as a replacement test for smear microscopy for diagnosing pulmonary TB in countries with an intermediate or high burden of TB. However, the Xpert MTB/RIF assay should remain the preferred diagnostic for all persons suspected of having TB, providing there are sufficient resources and infrastructure to support its use, given the evidence, its ability to simultaneously identify rifampicin resistance and because it is automated.

- Several operational issues accompany the implementation of TB-LAMP: the needs for electricity, adequate storage and waste disposal, stock monitoring, and temperature control in storage settings where temperatures exceed the manufacturer's recommendation (currently 30 °C for TB-LAMP).
- TB-LAMP is designed and has been evaluated to detect *M. tuberculosis* in sputum specimens. Its use with other samples (e.g., urine, serum, plasma, cerebrospinal fluid or other body fluids) has not been adequately evaluated.
- Adoption of TB-LAMP does not eliminate the need for smear microscopy, which should be used for monitoring the treatment of patients with drug-susceptible TB. However, the demand for conventional sputum microscopy may decrease in settings where TB-LAMP fully or partially replaces conventional sputum microscopy.
- TB-LAMP should not replace the Xpert MTB/RIF assay because the Xpert MTB/RIF assay simultaneously detects *M. tuberculosis* and rifampicin resistance; it is automated; and the procedure is relatively simple.
- In settings where the Xpert MTB/RIF assay cannot be implemented (e.g., owing to an inadequate electric supply, or excessive temperatures, humidity, or dust), TB-LAMP may be a plausible alternative.

10. Plans for disseminating WHO's guidance on TB-LAMP

This WHO guidance will be published online (http://www.who.int/tb/publications/lamp-diagnosis-molecular/en/) and disseminated through WHO's Global TB Department listserv to all WHO Regional and Country Offices and Member States, the Global Laboratory Initiative, the New Diagnostics Working Group of the Stop TB Partnership, and donors, technical agencies and other stakeholders.

11. Research needs

The current recommendations for the commercial TB-LAMP manual assay should not prevent or restrict further research on new TB diagnostics, especially point-of-care assays that can be used as close as possible to where patients access TB treatment. Additional studies using standardized protocols that include a high-quality culture reference standard (liquid culture results on at least two samples) are needed to better inform national TB programmes of the relative performance of TB-LAMP compared with the the Xpert MTB/RIF assay. Further operational research on TB-LAMP should focus on the following priorities:

- evaluating diagnostic algorithms in different epidemiological and geographical settings and patient populations;
- conducting more rigorous studies with higher quality reference standards (including multiple specimen types and extrapulmonary specimens) to improve confidence in specificity estimates;
- determining needs for training, and assessments of competency and quality;
- gathering more evidence on the impact on TB treatment initiation, morbidity and mortality;
- performing country-specific cost–effectiveness and cost–benefit analyses of targeted TB-LAMP use in different programmatic settings;
- meeting the Standards for reporting Diagnostic Accuracy Studies (known as STARD) for future studies (see http://www.equator-network.org/reporting-guidelines/stard/).

12. Annexes

Annex 1. References to studies for the systematic review of the diagnostic accuracy of TB-LAMP

Studies included in the review

1. Hang PT, Peter J, Mello FCQ, Parraga T, Nguyen TNL, Nabeta P, et al. Performance of the TB-LAMP diagnostic assay in reference laboratories – results from a multi-centre study (Brazil, Peru, South Africa, Vietnam). Submitted to the International Journal of Tuberculosis and Lung Disease, April 2016.
2. Gray CM, Katamba A, Narang P, Giraldo J, Zamudio C, Joloba M, et al. Feasibility and operational performance of TB LAMP in decentralized settings – results from a multi-centre study. Submitted to the Journal of Clinical Microbiology, April 2016.
3. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: India. Unpublished.
4. N'guessan K, Horo K, Coulibaly I, Adegbele J, Kouame-Adjei N, Seck-Angu H, et al. Comparison of pulmonary tuberculosis diagnosis by AFB detection, TB-LAMP and GeneXpert MTB/RIF in Ivory Coast. Unpublished (see the abstract in Annex 3).
5. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Madagascar. Unpublished.
6. Nliwasa M, MacPherson P, Chisala P, Kamdolozi M, McEwen K, Kaswaswa K. The accuracy of loop-mediated isothermal amplification (LAMP) assay for tuberculosis diagnosis in adults with chronic cough in Malawi. Submitted to PLOS ONE, March 2016.
7. Khalief MS, Doulla B, Mtunga DD, Adepoyibi T, Moriera R, Faulx D, et al. Evaluation of a loop-mediated isothermal amplification test kit for the diagnosis of pulmonary tuberculosis in the United Republic of Tanzania. Unpublished (see the abstract in Annex 3).
8. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Uganda. Unpublished.
9. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Viet Nam. Evaluation of Loopamp MTBC detection kit for diagnosis of pulmonary tuberculosis at peripheral laboratory in Vietnam. Unpublished (see the abstract in Annex 3).
10. Kaku T, Minamoto F, D'Meza R, Morose W, Boncy J, Bijou J, et al. Assessment of accuracy of LAMP-TB method for diagnosing tuberculosis in Haiti. Japanese Journal of Infectious Diseases, Published online: March 18, 2016. DOI: 10.7883/yoken.JJID.2015.519

Studies excluded from the review

1. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Cameroon. Unpublished.
2. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Cambodia. Unpublished.
3. Getahun M, Dagne Z, Yaregal Z, Aster HM, Shewki M, Abyot M, et al. The role of molecular diagnostic methods for diagnosis smear negative pulmonary TB and concordance of empirical TB treatment with confirmatory assays in Ethiopia. Unpublished.
4. Bojang AL, FS Mend, LD Tientcheu, J Otu, M Antonio, B Kampmann , et al. Comparison of TB-LAMP, GeneXpert MTB/RIF and culture for diagnosis of pulmonary tuberculosis in The Gambia. J Infect. 2016;72(3):332–7. doi: 10.1016/j.jinf.2015.11.011.

5. Reddy S, Ntoyanto S, Sakadavan Y, Reddy T, Mahomed S, Dlamini M, et al. Evaluation of the loop-mediated isothermal amplification assay for the detection of *Mycobacterium tuberculosis* in symptomatic tuberculosis patients attending a primary healthcare clinic in Durban, South Africa. Unpublished.
6. Study conducted under FIND LAMP Request for Applications Memorandum of Understanding: Mongolia. Unpublished.
7. Ou X, Li Q, Xia H, Pang Y, Wang S, Zhao B, et al. Diagnostic accuracy of the PURE-LAMP test for pulmonary tuberculosis at the county-level laboratory in China. PLOS ONE. 9(5):e94544. doi: 10.1371/journal.pone.0094544.

Annex 2. GRADE Evidence Profiles

Table 10. GRADE evidence profile: accuracy of TB-LAMP as a replacement test for sputum-smear microscopy for diagnosing pulmonary TB among all adults suspected of having pulmonary TB

Question: What is the diagnostic accuracy of TB-LAMP for to diagnose pulmonary tuberculosis in all adults with presumptive pulmonary TB compared with a culture reference standard?
Participants: Adult patients suspected of having TB
Prior testing: None

Role: Replacement test for sputum smear microscopy
Settings: Peripheral level laboratories
Index (new) test: TB-LAMP
Reference standard: Culture (Reference standard 1)
Studies: Mainly cross-sectional

TB-LAMP		smear microscopy	
Sensitivity	0.78 (95% CI: 0.71 to 0.83)	**Sensitivity**	0.63 (95% CI: 0.56 to 0.69)
Specificity	0.98 (95% CI: 0.96 to 0.99)	**Specificity**	1.00 (95% CI: 0.97 to 1.00)

Outcome	Nb of studies (Nb of patients)	Study design	Factors that may decrease the quality of evidence					Effect per 1000 patients tested				Test accuracy QoE
								Pre-test probability of 5%		Pre-test probability of 15%		
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	TB-LAMP	Smear microscopy	TB-LAMP	Smear microscopy	
True positives (patients with pulmonary TB)	7 studies 1 810 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	very serious[c]	not serious	none	39 (36 to 42)	32 (28 to 35)	117 (107 to 124)	95 (84 to 104)	**VERY LOW**
								7 more TP in TB-LAMP		**22 more TP in TB-LAMP**		
False negatives (patients incorrectly classified as not having pulmonary TB)								11 (8 to 14)	18 (15 to 22)	33 (26 to 43)	55 (46 to 66)	
								7 fewer FN in TB-LAMP		**22 fewer FN in TB-LAMP**		
True negatives (patients without pulmonary TB)	7 studies 1 810 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	serious[d]	not serious	none	932 (909 to 942)	948 (923 to 950)	834 (813 to 843)	848 (826 to 850)	**VERY LOW**
								16 fewer TN in TB-LAMP		**14 fewer TN in TB-LAMP**		
False positives (patients incorrectly classified as having pulmonary TB)								18 (8 to 41)	2 (0 to 27)	16 (7 to 37)	2 (0 to 24)	
								16 more FP in TB-LAMP		**14 more FP in TB-LAMP**		

CI: confidence interval; RFA, request for application.
a. The QUADAS-2 tool was used to asses the risk of bias. One study performed only Löwenstein–Jensen culture (Madagascar RFA); six studies used mycobacterial growth indicator tube liquid culture (BACTEC MGIT, Becton Dickinson, Franklin Lakes, NJ, United States) and had culture contamination rates < 5%; two studies (Uganda RFA, Haiti unpublished) did not exclude all participants with prior TB; three studies (Madagascar RFA, Uganda RFA, Haiti unpublished) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
b There were serious concerns about applicability because no studies were conducted in peripheral microscopy centres (four were performed at reference laboratories and three were performed at outpatient clinics affiliated with a hospital or university). The evidence was downgraded by one point.
c There was considerable heterogeneity in sensitivity estimates across individual studies. The evidence was downgraded by one point.
d There was moderate heterogeneity in specificity estimates across individual studies. The evidence was not further downgraded.

Table 11. GRADE evidence profile: accuracy of TB-LAMP as a replacement test for sputum-smear microscopy for diagnosing pulmonary TB among all adults living with HIV suspected of having pulmonary TB

Question: What is the diagnostic accuracy of TB-LAMP for to diagnose pulmonary tuberculosis in adults with HIV suspected of having pulmonary TB compared with a culture reference standard?
Participants: HIV positive adult patients with presumptive TB
Prior testing: None

Role: Replacement test for smear microscopy
Settings: Peripheral level laboratories
Index (new) test: TB-LAMP
Reference standard: Culture (Reference standard 2)
Studies: Mainly cross-sectional

TB-LAMP		smear microscopy	
Sensitivity	0.64 (95% CI: 0.49 to 0.76)	**Sensitivity**	0.62 (95% CI: 0.34 to 0.89)
Specificity	0.99 (95% CI: 0.85 to 1.00)	**Specificity**	0.99 (95% CI: 0.95 to 1.00)

Outcome	Nb of studies (Nb of patients)	Study design	Factors that may decrease the quality of evidence					Effect per 1000 patients tested				Test accuracy QoE
								Pre-test probability of 5%		Pre-test probability of 15%		
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	TB-LAMP	Smear microscopy	TB-LAMP	Smear microscopy	
True positives (patients with pulmonary TB)	4 studies 271 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	not serious	serious[c]	none	32 (25 to 38)	31 (17 to 45)	96 (74 to 114)	93 (51 to 134)	**VERY LOW**
								1 more TP in TB-LAMP		**3 more TP in TB-LAMP**		
False negatives (patients incorrectly classified as not having pulmonary TB)								18 (12 to 25)	19 (5 to 33)	54 (36 to 76)	57 (16 to 99)	
								3 fewer FN in TB-LAMP		**3 fewer FN in TB-LAMP**		
True negatives (patients without pulmonary TB)	4 studies 271 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	not serious	serious[c]	none	939 (808 to 949)	941 (903 to 950)	840 (722 to 849)	848 (826 to 850)	**VERY LOW**
								2 fewer TN in TB-LAMP		**2 fewer TN in TB-LAMP**		
False positives (patients incorrectly classified as having pulmonary TB)								11 (1 to 142	9 (0 to 47)	10 (1 to 128)	8 (0 to 42)	
								2 more FP in TB-LAMP		**2 more FP in TB-LAMP**		

CI: confidence interval; EVAL: evaluation study conducted in a reference laboratory; RFA: request for application.
a The QUADAS-2 tool was used to asses the risk of bias. There were insufficient studies to obtain pooled estimates using reference Standard 1; two studies used mycobacterial growth indicator tube liquid culture (BACTEC MGIT, Becton Dickinson, Franklin Lakes, NJ, United States) and had culture contamination rates < 5% (South Africa EVAL, Uganda RFA); two studies (Uganda RFA, United Republic of Tanzania RFA) did not exclude participants with prior TB; one study (Malawi RFA) excluded < 20% of eligible participants because of insufficient culture data for reference Standard 2; and two studies (Uganda RFA, United Republic of Tanzania RFA) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
b There were serious concerns about applicability because only one study was conducted in a peripheral microscopy centre. One study was performed in a reference laboratory and two studies were performed in outpatient clinics affiliated with a hospital or university. The evidence was downgraded by one point.
c There were small sample sizes and wide confidence intervals for the pooled estimates. The evidence was downgraded by one point for imprecision.

Table 12. GRADE evidence profile: accuracy of TB-LAMP as an add-on test following negative sputum-smear microscopy for diagnosing pulmonary TB in adults suspected of having pulmonary TB

Question: What is the diagnostic accuracy of TB-LAMP as an add-on test following a negative sputum smear microscopy to diagnose tuberculosis in adults suspected of having pulmonary TB?
Participants: Adult patients suspected of having sputum smear negative pulmonary TB
Prior testing: Smear microscopy

Role: Add-on test in the further testing of sputum smear negative persons
Settings: Peripheral level laboratories
Index (new) test: TB-LAMP
Reference standard: Culture (Reference standard 1)
Studies: Mainly cross-sectional

Sensitivity	0.42 (95% CI: 0.30 to 0.55)
Specificity	0.98 (95% CI: 0.96 to 0.99)

Outcome	Nb of studies (Nb of patients)	Study design	Factors that may decrease the quality of evidence					Effect per 1000 patients tested		Test accuracy QoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	Pre-test probability of 5%	Pre-test probability of 15%	
True positives (patients with pulmonary TB)	7 studies 1 349 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	very serious[c]	not serious	none	21 (15 to 28)	63 (45 to 83)	**VERY LOW**
False negatives (patients incorrectly classified as not having pulmonary TB)								29 (22 to 35)	87 (67 to 105)	
True negatives (patients without pulmonary TB)	7 studies 1 349 patients	Cross-sectional (cohort type accuracy study)	very serious[a]	serious[b]	serious[d]	not serious	none	931 (912 to 941)	833 (816 to 842)	**VERY LOW**
False positives (patients incorrectly classified as having pulmonary TB)								19 (9 to 38)	17 (8 to 34)	

CI: confidence interval; RFA: request for application.
a The QUADAS-2 tool was used to asses the risk of bias. One study performed only Löwenstein–Jensen culture (Madagascar RFA); six studies used mycobacterial growth indicator tube liquid culture (BACTEC MGIT, Becton Dickinson, Franklin Lakes, NJ, United States) and had culture contamination rates < 5%; two studies (Uganda RFA, Haiti unpublished) did not exclude all participants with prior TB; three studies (Madagascar RFA, Uganda RFA, Haiti unpublished) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
b There were serious concerns about applicability because no studies were conducted in peripheral microscopy centres (four were performed at reference laboratories and three were performed at outpatient clinics affiliated with a hospital or university). The evidence was downgraded by one point.
c There was considerable heterogeneity in sensitivity estimates across individual studies. The evidence was downgraded by one point.
d There was moderate heterogeneity in specificity estimates across individual studies. The evidence was not further downgraded.

Annex 3. Abstracts for unpublished studies

Included study 4. Comparison of pulmonary tuberculosis diagnosis by AFB detection, TB-LAMP and GeneXpert MTB/RIF in Ivory Coast

K. N'guessan,[1] K. Horo[2], I. Coulibaly,[3] J. Adegbele,[1] N. Kouame-Adjei,[3] H. Seck-Angu,[1] A. Guei,[1] J. Kouakou,[4] M. Dosso[1]

1 Laboratoire Nationale de Référence de la Tuberculose, Institut Pasteur de Côte d'Ivoire, 01BP 490 Abidjan 01
2 Service de Pneumologie CHU de Cocody
3 Centre Antituberculeux de Yopougon
4 Programme National de Lutte contre la Tuberculose.

Background: The specificity of acid-fast microscopy is excellent for mycobacterial species, but its sensitivity is not optimal. Unfortunately, it remains the main tool for pulmonary tuberculosis diagnosis in low-income countries. Early diagnosis and appropriate treatment are important for tuberculosis control. This study aimed to determine the performance of acid-fast bacilli detection after Ziehl–Neelsen staining, the loop-mediated amplification test for tuberculosis (TB-LAMP) and the GeneXpert MTB/RIF assay in symptomatic patients versus culture in liquid medium.

Methods: Sputum samples were collected from patients recruited at CAT de Yopougon (intermediate-level tuberculosis laboratory). Using the same sample, direct examination after Ziehl–Neelsen staining and TB-LAMP were performed by technicians blinded to the results of the other test. Samples were then transported to the Institut Pasteur de Côte d'Ivoire in an icebox at 4 °C and decontaminated according to the NALC method. After centrifugation, the pellet was used for testing by MGIT culture and the GeneXpert MTB/RIF assay.

Results: Of the 500 patients enrolled, 469 were included. Clinical isolates of *M. tuberculosis* complex were detected for 157 (33.5%). Compared with culture, the sensitivity and specificity of direct examination were, respectively, 86% (95% CI : 81–91) and 96% (95% CI : 94–98). Sensitivity of the GeneXpert MTB/RIF assay was 96% (95% CI : 0.93–0.99) and for TB-LAMP it was 92% (95% CI: 0.88–0.96). Specificity of the molecular method was 90% (95% CI: 87–93) for Xpert MTB/RIF and 94% (95% CI: 91–97) for TB-LAMP. In total, 147 (31.3%), 162 (34.5%), 183 (39%) active pulmonary TB cases were detected, respectively, by smear examination, TB-LAMP and GeneXpert MTB/RIF.

Conclusion: Compared with microscopy, molecular methods increased TB cases diagnosed by at least 3.2%.

Keywords: Diagnosis; Ziehl–Neelsen TB-LAMP; GeneXpert; Tuberculosis

Included study 7. Evaluation of a loop-mediated isothermal amplification test kit for the diagnosis of pulmonary tuberculosis in the United Republic of Tanzania

Mbarouk Seif Khalief,[1] Basra Doulla,[2] Daphne Dan Mtunga,[2] Tope Adepoyibi,[3] Rick Moriera,[3] Dunia Faulx,[4] Timothy Chonde,[5] Edgar Luhanga,[5] Beatrice Mutayoba,[6] Blasdus Njako,[6] David S. Boyle[5]

1 Amana Regional Referral Hospital, P.O. Box 5368, Dar es Salaam, United Republic of Tanzania
2 Ministry of Health and Social Welfare, P.O. Box 9083, Dar es Salaam, United Republic of Tanzania
3 The Burnett Institute, 5 Commercial Road, Melbourne, Victoria, Australia 3004
4 PATH, 2201 Westlake Avenue, Suite 200, Seattle, WA 98121, USA
5 PATH, P.O. Box 13600, Dar es Salaam, United Republic of Tanzania
6 National TB and Leprosy Programme, 10th Floor, Extelecom Building, Samora Avenue, P.O. Box 9083, Dar es Salaam, United Republic of Tanzania
Corresponding author: dboyle@path.org, (+11) 206 302 4748.

Background: The Eiken Loopamp™ MTBC loop-mediated isothermal amplification (LAMP) test kit is proposed to provide a more accurate and convenient alternative to sputum-smear microscopy for the detection of pulmonary tuberculosis (TB) in microscopy centres in resource-constrained settings. This study is the first to evaluate the clinical accuracy and operational features of the redesigned Eiken Loopamp test kit for the detection of pulmonary TB in a clinical setting in Africa.

We compared the performance of the LAMP assay with fluorescence sputum-smear microscopy (SSM) and Xpert MTB/RIF. A composite gold standard of solid and liquid culture was used to confirm TB-positive and -negative patients.

Methods and Findings: From November 2013 to May 2014, 650 consenting individuals older than 18 years of age with presumptive pulmonary TB were tested for TB with LAMP, SSM and Xpert MTB/RIF. After data review, a sample set of 550 participants was used. As compared with culture, the sensitivity and specificity of the Loopamp assay were, respectively, 67.4% and 97.5%. The performance of other tests on the same specimen had sensitivity and specificity of 80% and 90.3% (GeneXpert) and 61.4% and 96.3% (SSM); the the sensitivity and specificity of using two SSM test results was 71.43% and 95.47%.

Conclusion: A low-cost yet accurate and robust platform is needed to replace smear microscopy in the diagnosis of pulmonary TB. Our data show that while the Loopamp assay has greater sensitivity when compared with a single SSM test, the sensitivity of two SSM tests is greater. Therefore, as evaluated under this setting, the Loopamp assay does not offer an improvement in the diagnosis of pulmonary tuberculosis as compared with current SSM methods.

Included study 9. Evaluation of Loopamp MTBC detection kit for diagnosis of pulmonary tuberculosis at peripheral laboratory in Vietnam

Thanh Hoa Thi Tran,[1] Hung Van Nguyen,[2], Xuyen Thi Le,[2] Thuong Van Dinh,[3] Ha Hoang Du,[2] Chinh Van Dao,[4] Viet Nhung Nguyen,[2] Van Anh Thi Nguyen[1]
1 Department of Bacteriology, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam
2 Department of Microbiology, National Lung Hospital, Hanoi, Viet Nam
3 Tuberculosis unit, Ung Hoa District Health Centre, Hanoi, Viet Nam
4 Ha Dong centre for Tuberculosis and Lung Diseases, Hanoi, Viet Nam

Tuberculosis (TB) is one of the most dangerous transmissible diseases threatening public health worldwide. Early and effective detection of TB patients at peripheral laboratories in TB high-prevalence settings is a priority for global TB control. The TB detection test should be rapid, specific and have the potential to replace the conventional smear microscopy test. This study aimed to evaluate the LoopampTM MTBC detection kit, which uses loop-mediated isothermal amplification for TB (TB-LAMP), at a peripheral laboratory in Viet Nam.

From January 2014 to April 2014, 503 people with TB typical symptoms were consecutively enrolled from the Ung Hoa district, Hanoi, Viet Nam. Three sputum samples (two spots and one morning) were collected from each of these presumptive cases. The results of a single TB-LAMP test done at the district health centre laboratory were compared with three smear microscopy and a single GeneXpert MTB/RIF tests, using single culture as the reference test. Test results were available from 498 subjects with a mean (SD) age of 58.6 (14.9) years, of which 282 were males and 216 were females. The results showed that the sensitivity of TB-LAMP was 80% (95% CI: 51.9–95.7%) in smear-positive/culture-positive samples and 15.8% (95% CI: 33.8–39.6%) in smear-negative/culture-positive samples. The TB-LAMP kit had overall sensitivity of 44.1% (15/34; 95% CI: 27.2–62.1%) and specificity of 95.0% (441/464; 95% CI: 94.2–97.9%). All the accuracy parameters of TB-LAMP were lower than those of the Xpert MTB/RIF assay and smear microscopy. Whilst Xpert MTB/RIF and smear microscopy displayed a high and fair correlation to culture, the agreement between TB-LAMP and culture was low (kappa coefficient was 0.87, 0.53 and 0.37 for Xpert MTB/RIF, smear microscopy and TB-LAMP). Although TB-LAMP specificity was lower than that of smear microscopy (95.1% vs. 98.9%), the single TB-LAMP test had sensitivity equal to that of three smear microscopy tests and was higher than any single smear microscopy result. The LoopampTM MTBC detection kit, therefore, could be a possible replacement for acid-fast bacilli smear microscopy for acid-fast bacilli at peripheral laboratories in areas with a high prevalence of TB. However, the kit should be improved in terms of its technology, design and implementation procedure in order to increase its sensitivity and specificity when being applied at this primary level.

THE
END TB
STRATEGY

World Health Organization

For further information, contact:
Global TB Programme
World Health Organization
Avenue Appia 20, CH-1211
Geneva-27, Switzerland
Information Resource Centre HTM/GTB:
Email: tbdocs@who.int
Website: www.who.int/tb

9 789241 511186