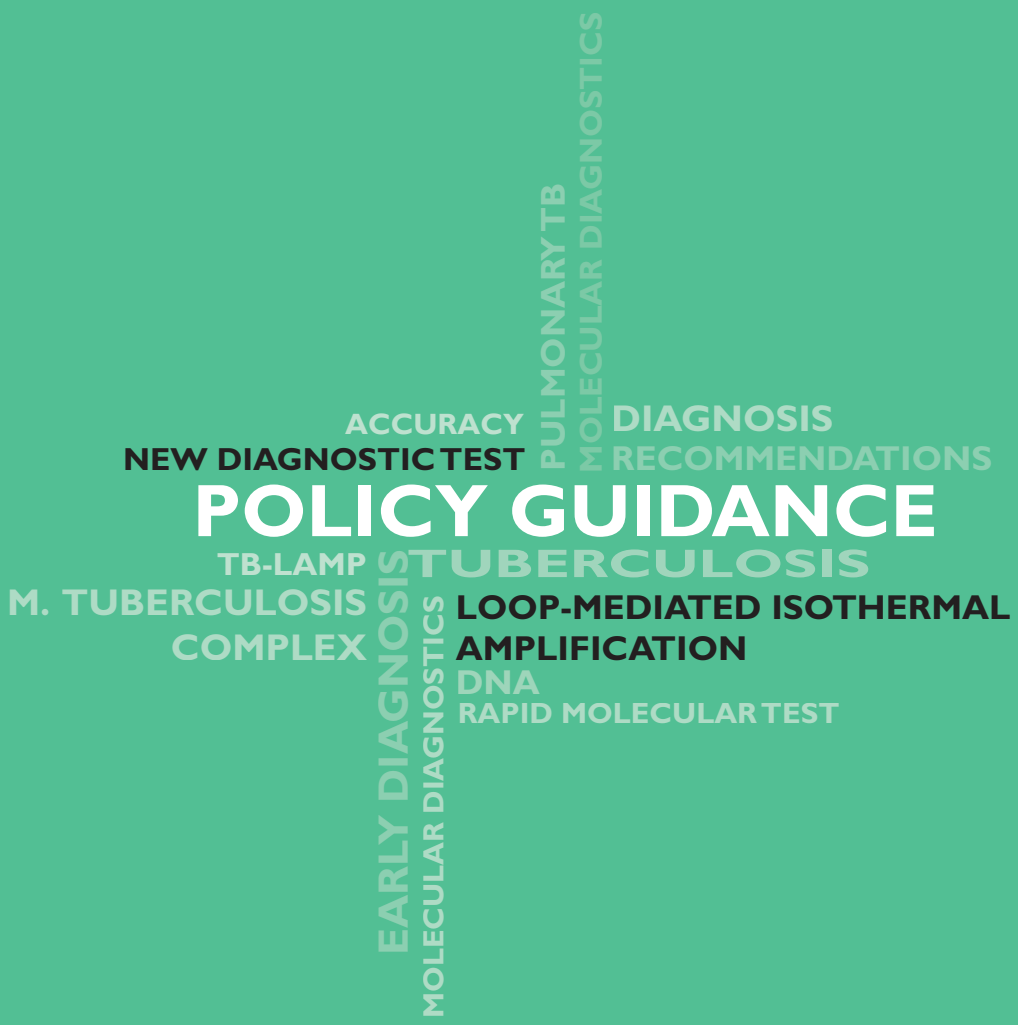
The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis



THE END TB STRATEGY

World Health Organization

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

Policy guidance

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Abbreviations

AFB	acid-fast bacilli
CI	confidence interval
CRS	composite reference standard
DOI	Declaration of Interests
DST	drug-susceptibility testing
GDG	guideline development group
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HIV	human immunodeficiency virus
LAMP	loop-mediated isothermal amplification
MDR-TB	multidrug-resistant tuberculosis
NAAT	nucleic acid amplification test
PICO	Population, Intervention, Comparator, Outcome
PLHIV	People living with HIV/AIDS
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
TB	tuberculosis
TB-LAMP	loop-mediated isothermal amplification for detection of *Mycobacterium tuberculosis*
USAID	United States Agency for International Development
WHO	World Health Organization

Declarations and management of conflicts of interests

The members of the Guideline Development Group (GDG), the systematic review team and members of the External Review Group completed Declarations of Interests (DOIs). These were reviewed by the WHO Steering Group prior to the meeting of the GDG in January 2016. The review of each DOI assessed 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 DOIs of the GDG members were declared significant or potentially significant. Two individuals declared significant interests and were designated observers to the webinar. Members of the systematic review team were invited to provide technical input and answer technical questions. The observers and the systematic review authors did not participate in the GRADE evaluation process nor in the final discussions when recommendations were developed. Also, they were not involved in developing the report of the GDG meeting, nor in preparing the WHO policy guidance.

The following interests were declared:

None declared

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

Declared, insignificant

Wendy Stevens declared that she had received remuneration for performing other TB assay validations (Cepheid, Abbott, Roche, Hain, DNA genotek, Alere) generally in the form of reagents. No funding related to TB-LAMP.

Anna Vassall declared that she had performed a consultancy to model the cost-effectiveness of new diagnostics.

Karen Steingart declared that she had performed systematic reviews for the use of on GenoType® MTBDRsl and urine LF-LAM assays.

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

Daniela Maria Cirillo declared that she had received research grants for the evaluation of new TB test under development from FIND and Italian Government (EUR 17,000). No funding related to TB-LAMP was declared.

Declared, significant (Observer status)

Satoshi Mitarai declared presenting at an Eiken sponsored symposium at the Union Conference Cape Town in December 2015. He declared research support including paid travel to meeting (5th National LAMP research forum, China)

Beatrice Mutayoba was the co-investigator for the TB-LAMP evaluation study in Tanzania

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

Executive summary

Background

The WHO End TB Strategy calls for the early diagnosis of tuberculosis (TB) and universal drug susceptibility testing (DST), highlighting the critical role of laboratories in the post-2015 era for rapidly and accurately detecting TB and drug resistance. Molecular assays based on nucleic acid amplification techniques such as polymerase chain reaction (PCR) have been developed for rapid TB diagnosis and are being implemented in developing countries. Loop-mediated isothermal amplification (LAMP) is a unique temperature-independent way of DNA amplification, and is facilitated by a visual optic readout in an instrument that is robust and can be used at the peripheral health center level where microscopy is performed. LAMP methodology has been used for the detection of malaria and several neglected tropical diseases.

A commercial molecular assay based on loop-mediated isothermal amplification (TB-LAMP) was developed by Eiken Chemical Company Ltd (Tokyo, Japan) for the detection of *Mycobacterium tuberculosis* complex (MTBC). TB-LAMP is a manual assay that requires less than one 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 available at that time, and found that TB-LAMP technology had potential as a rapid TB diagnostic tool but recommended that additional studies be conducted [1]. Since 2012, 20 studies across 17 countries have been conducted. In January 2016, WHO convened a GDG via webinar to review the recent evidence for the TB-LAMP.

Objectives, rationale and methods used to develop the guidance

TB-LAMP has minimal laboratory infrastructure and biosafety requirements and has been evaluated as a rapid, point-of-care test as an alternative to sputum smear microscopy which remains the primary diagnostic test for pulmonary TB in resource-limited settings. This document provides a summary of the evidence and recommendations for the use of the commercial TB-LAMP assay (Loopamp™MTBC Detection Kit, Eiken Chemical Company Ltd., Japan) for the detection of *Mycobacterium tuberculosis* complex directly from sputum specimens from persons with signs and symptoms consistent with pulmonary TB.

The objectives for developing the guideline:

- Determine the diagnostic accuracy of TB-LAMP for detection of pulmonary TB compared with mycobacterial culture when used as a replacement test for sputum smear microscopy among all adults and among HIV-infected adults with signs and symptoms consistent with pulmonary TB;
- Determine the diagnostic accuracy of TB-LAMP for detection of pulmonary TB compared with mycobacterial culture when used as an add-on test following a negative sputum smear microscopy among adults with signs and symptoms consistent with pulmonary TB;
- Determine the difference in diagnostic accuracy between TB-LAMP and Xpert® MTB/RIF, Cepheid, Sunnyvale Ca., USA (Xpert MTB/RIF) for detection of pulmonary TB compared with mycobacterial culture among all adults with signs and symptoms consistent with pulmonary TB;
- Determine the proportion of indeterminate/invalid results when TB-LAMP is used to detect pulmonary TB among all adults with signs and symptoms consistent with pulmonary TB.

[1] WHO Expert Group Meeting Report. 2013. The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis. WHO/HTM/TB/2013.05

The systematic review authors identified all published and unpublished studies of TB-LAMP that were conducted using the modified design-locked assay protocol from January 2012. Standardized eligibility criteria were applied to select individual studies and study participants for the analysis. The quality of included studies was assessed using the QUADAS-2 tool.

The WHO policy recommendations developed from the evidence synthesis process by the Guideline Development Group are summarized below.

WHO's policy recommendations

1. TB-LAMP may be used as a replacement test for sputum smear microscopy for the diagnosis of pulmonary TB in adults with signs and symptoms consistent with TB (Conditional recommendation, Very low quality of 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 of evidence).

Remarks

- These recommendations apply to settings where conventional sputum smear microscopy is able to 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 MDR-TB;
- Due to the limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over sputum smear microscopy for the testing of persons living with HIV with 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 the use of the TB-LAMP assay in children, based on the generalization of data in adults, while acknowledging difficulties in the collection of sputum specimens from children.

1. Background

Tuberculosis (TB) remains a large-scale public health problem. Key global priorities for tuberculosis (TB) care and control include improving case-detection and detecting patients earlier, including patients with smear-negative TB disease. In 2014, only 63% (6 million) of an estimated 9.6 million people developed TB were reported to WHO, meaning that globally 37% of estimated TB cases are left undetected. The WHO has identified the development and evaluation of new diagnostic tools as a necessary part of further efforts[2].

The WHO End TB Strategy calls for the early diagnosis of TB and universal drug susceptibility testing (DST), highlighting the critical role of laboratories in the post-2015 era for rapidly and accurately detecting TB and drug resistance. Molecular assays based on nucleic acid amplification techniques such as polymerase chain reaction (PCR) have been developed for rapid TB diagnosis and are being implemented in developing countries. A commercial molecular assay Loopamp™ MTBC Detection Kit based on loop-mediated isothermal amplification was developed by Eiken Chemical Company Ltd (Tokyo, Japan) for the detection of *Mycobacterium tuberculosis* complex (TB-LAMP). TB-LAMP is a manual assay that requires less than two hours to perform and can be read with the naked eye under ultra violet light. Because of its limited infrastructure requirements and relative ease of use, TB-LAMP is being explored as a rapid, point-of-care diagnostic test for resource-limited settings. LAMP methodology has been used for the detection of malaria and several neglected tropical diseases.

In 2012, WHO convened a Guideline Development Group (GDG) which recognized that TB-LAMP offers a manual molecular approach to TB detection that seems to be feasible to implement in peripheral microscopy laboratories once laboratory technicians have undergone adequate training[3,4]. TB-LAMP has the advantages of being relatively high-throughput, does not require sophisticated instrumentation, and has the biosafety requirements similar to those for performing sputum smear microscopy.

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

The evidence reviewed and this policy guidance applies to the use of the commercial TB-LAMP assay only. Other DNA-based assays for the detection of *Mycobacterium tuberculosis* complex (MTBC) were not evaluated. Any new or generic assay to detect the presence of MTBC using LAMP or other DNA amplification method should be subject to adequate evaluation and validation in the settings of intended use as per WHO policy[5].

Index test

The fundamental amplification reaction requires four types of primers which are complementary to six regions of the target gene (Figure 1). As double-stranded DNA is in a condition of dynamic equilibrium at a temperature 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

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[2] WHO 2015. World Health Organization. Global tuberculosis control: WHO report 2015. WHO/HTM/TB/2015.22

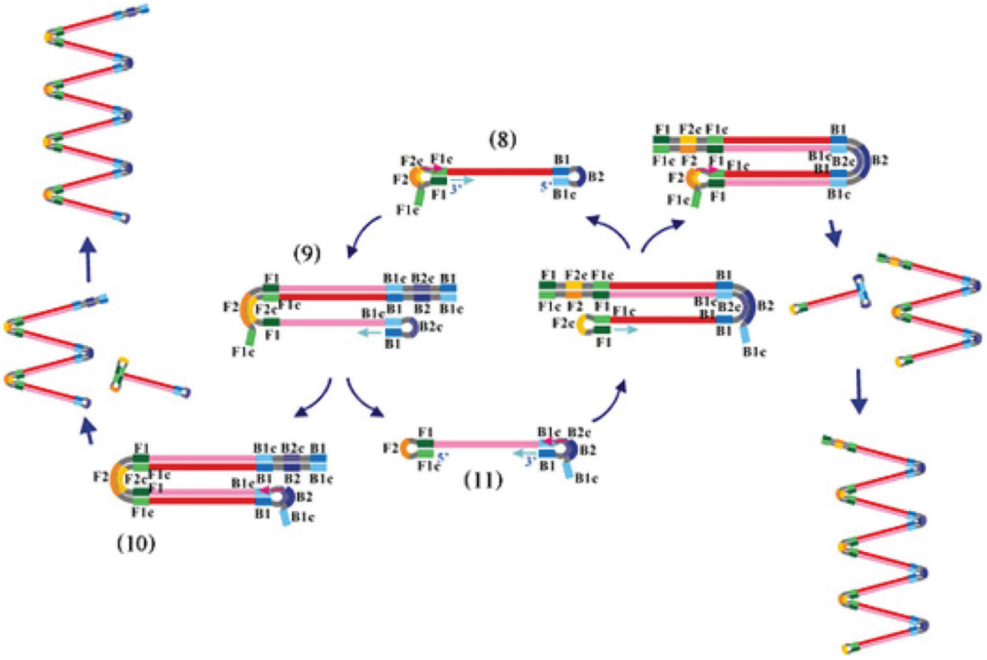
[3] WHO Expert Group Meeting Report. 2013. The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis. WHO/HTM/TB/2013.05

[4] Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, Sanga E, Hoelscher M, Notomi T, Hase T, Perkins MD. 2007. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol 45:1936–40.

[5] WHO 2015. Implementing tuberculosis diagnostics. Policy Framework. WHO/HTM/TB/2015.11 http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf?ua=1

polymerase with strand displacement activity displacing and releasing a single-stranded DNA. Due to the complementarity of the 5' end of the FIP and BIP primers in nearby regions of the target amplicon, loop structures are formed. This allows various sized structures consisting of alternately inverted repeats of the target sequence on the same strand to be formed in rapid succession.

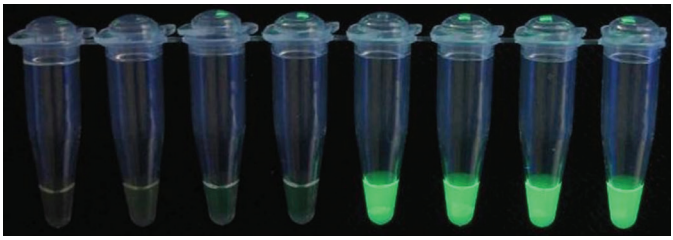
Figure 1. TB-LAMP molecular principle



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 10^9 - 10^{10} times can be achieved within 15-30 minutes. The TB-LAMP assay includes loop primers for a total of six primers binding to 8 locations. This requirement for homogeneous sequence 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 visible detection of successful amplification possible by using dsDNA-binding dyes such as SYBR green, by detecting turbidity caused by precipitating magnesium pyrophosphate, or by using a non-inhibitory fluorescing reagent that is quenched in the presence of divalent cations. In the picture below, calcein, unquenched by pyrophosphate consumption of divalent cations, fluoresces under UV light. The turbid, fluorescent product is easily seen with the naked eye (Figure 2).

Figure 2. Visual readout of TB-LAMP results applying ultraviolet (UV) light



The evaluated version of TB-LAMP consisted of the following steps (Figure 3):

1. Sample preparation (10 – 20 min):

- Using a wide-bore disposable pipette, collect 60 µL of sputum from a specimen container and transfer the collected sputum to a heating tube containing extraction solution.
- Mix by inverting 3-4 times and place the heating tube in heating block at 90°C for 5 min to lyse and inactivate mycobacteria.
- Remove the heating tube from the heating block and let it cool down for 2 minutes.
- Attach the heating tube to an adsorbent tube and mix by shaking until all the powder has completely mixed with the solution.
- Place an injection cap on the adsorbent tube and screw tightly to pierce the seal.
- Insert the nozzle into a reaction tube and transfer drops of solution (30 µl) to the reaction tube.

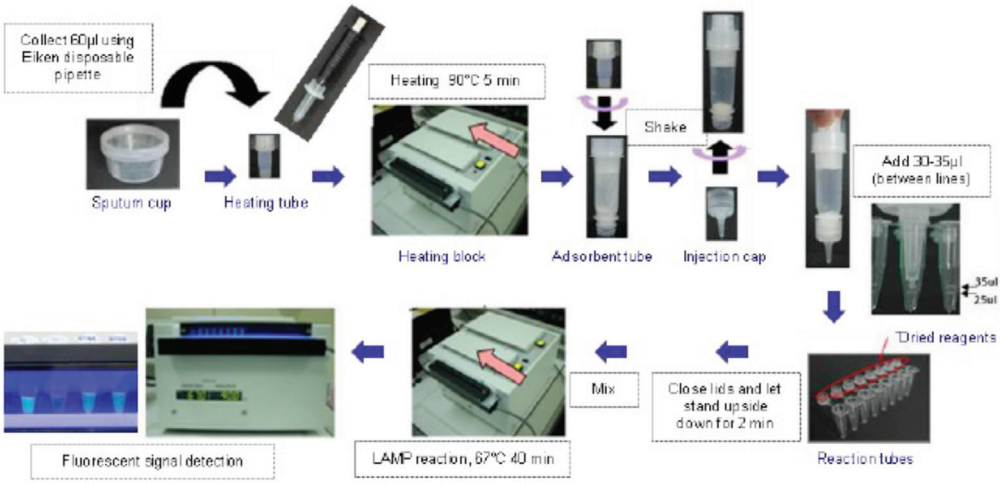
2. Amplification (40 min):

- Confirm the temperature on the digital display on the incubator to be 67°C.
- Load the reaction tubes into the heating block and start the reaction.
- The amplification is stopped automatically after 40 min.

3. Visual detection of fluorescence light from the reaction tube using UV light (0.5 – 1 min):

- Transfer the reaction tubes into the fluorescence detector and record the results.
- Discard reaction tubes (without opening the tubes).
- Dispose of waste as per National Guidelines

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



2. Methods

2.1. Evidence synthesis

In accordance with WHO standards for assessing evidence when formulating policy recommendations, the GRADE approach (the 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 the impact on patient- and public health of new diagnostic tests.

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 Guideline Development Group. PICO refers to the following four elements that should be included in questions that govern a systematic search of the evidence: the **P**opulation targeted by the action or intervention (in the case of systematic reviews of diagnostic test accuracy, P is the population of interest); the **I**ntervention (I is the index test; the **C**omparator (C is the comparator test(s); and the **O**utcome (O is usually sensitivity and specificity). The PICO questions for the review are given below.

This systematic review addresses the following questions framed in the "Population/Intervention/Comparison/Outcome" (PICO) style recommended for evidence-based medicine:

PICO questions addressed by the GDG

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

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

3. What is the difference in diagnostic accuracy between TB-LAMP and Xpert MTB/RIF for detection of pulmonary TB in reference to mycobacterial culture among all adults?

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

2.2. Criteria for considering studies for this review

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

Studies were excluded if they did not exclude patients on 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 prior history of TB, were less than 18 years of age, did not have results of rapid speciation testing for MTB available or who had a positive mycobacterial culture but speciation testing was negative suggesting the presence of non-tuberculous mycobacteria (NTM).

Individual participants were also excluded if the TB-LAMP assay was performed on samples other than sputum or if the total reaction volume was less than 25 µL. For the comparison of TB-LAMP to the Xpert MTB/RIF assay, individual participants were excluded if Xpert MTB/RIF was performed on frozen specimens or both valid TB-LAMP and Xpert MTB/RIF results 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 from analysis.

The following mycobacterial culture reference standards were used to classify TB status. Eligible studies performed one or more sputum cultures on solid media (Lowenstein-Jensen), liquid media (MGIT) or both. To account for the different number of cultures performed by studies and culture results available for study participants, three hierarchical culture-based reference standards were used to assess diagnostic accuracy:

Standard 1:

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

Standard 2:

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

Standard 3:

- TB: at least one positive culture confirmed to be MTB complex 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/participants included in the analysis (lowest with Standard 1 and highest with Standard 3). Thus, the potential for false-negative index test results is highest and false-positive index test results lowest with Standard 1. In contrast, the number of studies/study participants included is expected to be lowest with Standard 1, which excludes studies that performed only one culture and study participants for whom only one negative culture result is available due to culture contamination, and highest with Standard 3.

Studies recorded TB-LAMP and Xpert MTB/RIF results as negative, positive or indeterminate/invalid in accordance with manufacturer recommendations. Sputum smear microscopy varied across studies in terms of 1) Type of microscopy (one or more of direct Ziehl-Neelsen [ZN], concentrated ZN, direct fluorescence microscopy [FM] and concentrated FM); 2) Number of sputum specimens examined (one, two or three specimens) and 3) Number of smears prepared from each specimen examined (one or two smears). All studies recorded semi-quantitative microscopy results in accordance with WHO guidelines[6]. Microscopy results were standardized across studies by 1) Considering only direct ZN and direct FM results; 2) Considering only the first two smear results if more than two direct ZN or direct FM results were available; and 3) Defining patients to be sputum smear-positive if at least one acid-fast bacillus (AFB) was seen in any sputum smear.

[6] Lumb R, Van Deun A, Bastian I, Fitz-Gerald M. Laboratory Diagnosis of Tuberculosis by Sputum Microscopy: The Handbook. Global Laboratory Initiative. Adelaide: 2013. http://www.stoptb.org/wg/gli/assets/documents/TB%20MICROSCOPY%20HANDBOOK_FINAL.pdf

2.3. Data collection and analysis

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

For each study the minimum data required for each individual enrolled was:

- Age
- Smear microscopy results including semi-quantitative scoring
- Culture results (positive, negative, or contaminated)
- TB-LAMP results (positive, negative, or invalid)
- Study work flow

Additionally, where available:

- HIV status
- Sample collection time (spot or morning)
- Culture days to positivity for MGIT or quantitative scoring for LJ
- Species identification test results (MTB or NTM)
- TB-LAMP final reaction volume (< 25 µl, 25-35 µl, or > 35 µl)
- Operator that performed TB-LAMP
- Xpert® MTB/RIF test results (when performed; positive, negative, indeterminate)
- Drug susceptibility test results (conventional or LPA)

Data was extracted from Microsoft Excel sheets, SAS, EpiData, and Microsoft Access, depending on the study, to standardized data columns in Microsoft Excel.

Using the GRADE framework, calculations of test sensitivity and specificity were used as proxy measures for patient 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 delayed initiation of effective treatment, the development of more severe disease, morbidity, mortality and further transmission of disease. Poor specificity would result in *false-positive* results so that patients without TB would be prescribed unnecessary treatment which may have increased adverse effects.

Rates for true positives, true negatives, false positives and false negatives were calculated using 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 benefit of reassurance and alternative diagnosis;

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

The sensitivity and specificity of TB-LAMP, smear microscopy and Xpert MTB/RIF in each study were calculated with each mycobacterial culture-based reference standard. The proportion of indeterminate/invalid TB-LAMP results was calculated as the number indeterminate/invalid over the total number of patients eligible for the analysis of TB-LAMP accuracy among all adults. For all review questions, heterogeneity was assessed visually with forest plots and statistically with χ^2 and I^2 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 for which thirteen studies conducted in eleven countries met the inclusion criteria. The guideline methodologist participated in the initial planning, scoping and development of the key questions for the GDG meeting but was not available to participate in the GDG meeting.

The WHO Steering Group was responsible for scoping the guideline, drafting the PICO questions and overseeing the evidence retrieval and analyses. The Steering Group was also responsible for selecting members for the GDG and External Review Group, for managing declarations of interest, and for organising the GDG meetings. A brief biography of each of the GDG member was made available for public scrutiny on the WHO Global TB Programme website (http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/) two 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, i.e. unanimous agreement among all GDG members. Evidence to Recommendations tables were developed for the PICO questions.

The full set of evidence to recommendations tables are included in online annex 4. http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/

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 the Core Group members of the Global Laboratory Initiative Working Group. The External Review Group did not identify any major errors or missing data in the policy guidance. The External Review Group members confirmed that there were no concerns regarding any of the recommendations, any other setting-specific issues, nor any additional implications for implementation.

3. Scope

This document provides a pragmatic summary of the evidence and recommendations on the use of the TB-LAMP assay for the diagnosis of pulmonary TB in adults with signs and symptoms consistent with TB. It should be read in conjunction with the 2015 WHO 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 or drug-resistant TB and HIV. These documents are available at http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/

3.1. Target audience

This policy guidance is intended to be used by clinicians treating patients with TB, 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.

4. Evidence base for policy formulation

Of the 20 studies identified that evaluated TB-LAMP, 13 studies conducted in eleven countries met criteria for inclusion in the systematic review (Figure 4). Six studies were excluded as it was unclear whether participants had been on TB treatment within 60 days prior to enrolment. One study was excluded as rapid speciation testing on positive mycobacterial cultures was not performed. All 13 included studies provided individual-level data. Of the 13 included studies, 5 studies were conducted by FIND. These were four evaluation (EVAL) studies conducted in reference laboratories in Brazil, Peru, South Africa, and one demonstration (DEMO) study conducted in the setting of intended use and was performed in rural microscopy centers in India. Seven studies, each performed in a different country with independent protocols, were sponsored by FIND through a request for applications (RFA). One study in Haiti was conducted without any involvement by FIND but was sponsored by the manufacturer Eiken Chemical Company Ltd., Japan. For this study, the investigators were contacted and provided individual-level data.

The thirteen studies included in analysis involved 5099 participants and were conducted between January 2012 and December 2014. A total of 339 participants were excluded from the analysis. Of these, 22 participants had a documented history of prior TB, one participant had TB-LAMP testing performed on a non-sputum sample, 111 participants did not have results of speciation testing for MTB, 83 participants were under 18 years of age, and further 122 participants had TB-LAMP testing done with a total reaction volume less than 25 µL. Thus, 4760 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 and/or university-affiliated clinics, and three studies 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 and liquid) used for mycobacterial culture although all but one study (Madagascar RFA) performed at least one liquid culture. The liquid culture contamination rate was less than 5% for seven of twelve studies, including four in which the rate was 0%. In addition, all studies performed culture on sputum specimens that had been stored for one or more days. Participants in the majority of studies were predominately male and had a median age ranging from 33-60 years. Four of the thirteen included studies enrolled at least 10% HIV-positive participants (S. Africa EVAL, Malawi RFA, Uganda RFA, Ivory Coast RFA). The proportion of patients with culture-positive TB ranged from 20-40% for most studies, but was lower in three studies (India DEMO 11%, India RFA 15%, Vietnam RFA 8%) and higher in one study (Vietnam EVAL 66%). The proportion of patients with smear-negative TB varied widely, ranging from 13% (Ivory Coast RFA) to 59% (Vietnam RFA).

Figure 4. Study flow diagram for the 20 TB-LAMP studies identified through a systematic search. Thirteen studies were eligible for inclusion in the revised systematic review.

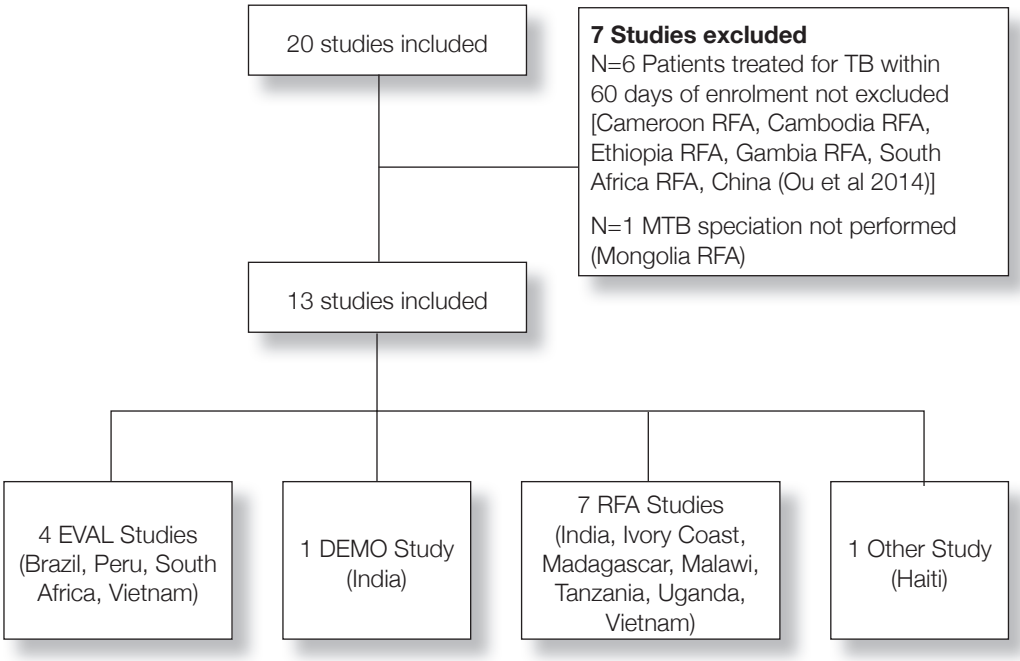


Table 1. Study characteristics for all included studies.

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

– Information not available

Abbreviations: TB, tuberculosis; MGIT, Mycobacterial Growth Indicator Tube; ZN, Ziehl-Neelsen; FM, fluorescence microscopy; LJ, Lowenstein-Jensen; EVAL, Evaluation study conducted in a reference laboratory; DEMO, Demonstration study conduct in the setting of intended use; RFA; Request for applications.

[1] HIV unknown counted as negative. Reflects proportion of study population known to be HIV positive

[2] Reference standard used for this calculation was Standard 3 for all studies

[3] Smear microscopy results based on analysis of 1 smear only

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[7]. 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. Signaling questions in each domain were used to form judgments 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 for 5 studies.

Table 2. Risk of bias and applicability concerns: review authors' judgments about each domain.

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
Tanzania (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	HIGH	LOW	LOW
Uganda (RFA)	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Ivory Coast (RFA)	LOW	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Madagascar (RFA)	LOW	LOW	HIGH	UNCLEAR	LOW	LOW	LOW
Haiti	UNCLEAR	LOW	HIGH	UNCLEAR	LOW	LOW	LOW

Patient selection. The risk of bias was judged to be "unclear" for 5 studies (India RFA, Tanzania RFA, Uganda RFA, Vietnam RFA, Haiti) because it could not be documented that patients with a history of TB 60 days prior to enrolment were excluded. Applicability concerns were judged to be "high" for 5 studies because they were conducted at a reference level laboratories (Brazil EVAL, Peru EVAL, South Africa EVAL, and Vietnam EVAL) or because enrolment involved screening of patients by a pulmonary specialist (Tanzania RFA). All studies enrolled patients consecutively and applied appropriate inclusion and exclusion criteria.

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 results of other tests. All studies used standard reporting of the result of the initial TB-LAMP test as negative, positive or indeterminate/invalid.

[7] 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. Annals of Internal Medicine 2011; 155(8):529-36.

Reference standard. The risk of bias was judged to be high for all studies because of failure to perform mycobacterial culture on at least two sputum samples (India DEMO, India RFA, Ivory Coast RFA, Malawi RFA, Tanzania RFA, Vietnam RFA), failure to use liquid culture (Madagascar RFA), or liquid culture contamination rates were outside the acceptable range of 5-12% (Brazil EVAL, Peru EVAL, South Africa EVAL, Vietnam EVAL, India RFA, Uganda RFA, Vietnam RFA and the Haiti study). All studies were judged to have low applicability concerns because TB culture is a recognised reference method for the bacteriological confirmation of TB.

Flow and timing. The risk of bias was judged to be high for one study (Malawi RFA) as over 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 7 studies (India RFA, Ivory Coast RFA, Madagascar RFA, Tanzania RFA, Uganda RFA, Vietnam RFA and Haiti study) due to lack of information regarding the percentage of eligible participants at each site who had data that was ultimately submitted for this analysis.

5. Systematic review results

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

Of 4760 adults eligible for inclusion in the analysis, 1810 participants (38%) across seven studies qualified for Standard 1, 3110 participants (65%) across ten studies for Standard 2, and 4596 participants (97%) across thirteen studies for Standard 3 (Table 3).

Table 3. TB-LAMP as a replacement test for smear microscopy: Eligible and included patients according to reference standard and study site.

Study	Total	Eligible[2]	Included		
			Standard 1[1]	Standard 2[1]	Standard 3[1]
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%)
Tanzania (RFA)	648	648	–	587 (91%)	632 (98%)
Uganda (RFA)	233	190	184 (97%)	189 (99%)	190 (100%)
Ivory Coast (RFA)	500	480	–	–	451 (94%)
Madagascar (RFA)	548	521	476 (91%)	476 (91%)	516 (99%)
Haiti	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 and 5 non-TB patients

[1] All reference standards classify patients as having TB if ≥1positive 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

[2] Total eligible includes missing and indeterminate TB-LAMP results: Brazil EVAL (n=2 indeterminate), South Africa EVAL (n=2 indeterminate), Malawi RFA (n=5 missing), Tanzania RFA (n=11 missing), Madagascar RFA (n=1 missing, n=1 indeterminate), Haiti (n=6 missing).

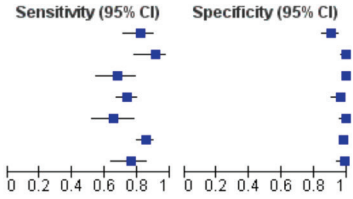
The sensitivity of TB-LAMP in individual studies ranged from 66-82% with Standard 1, 62-91% with Standard 2 and 48-100% with Standard 3 (Figure 5). There was significant heterogeneity in sensitivity estimates, both from visual inspection of forest plots and statistical testing (I^2 72%-94%, $p<0.003$ for all reference standards). Pooled sensitivity of TB-LAMP was higher than for smear microscopy, ranging from 77.7% (95% 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 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 reference standard used. The pooled sensitivity for the TB-LAMP among sputum smear-positive patients ranged from 95.2% (90.2-97.7) to 96.6% (91.9-98.6) across studies, depending on reference standard used.

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

Figure 5. Forest plots of TB-LAMP sensitivity and specificity for the detection of TB compared with three culture-based reference standards.

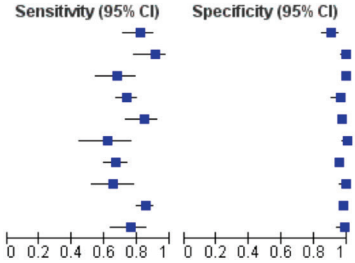
Standard 1

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]



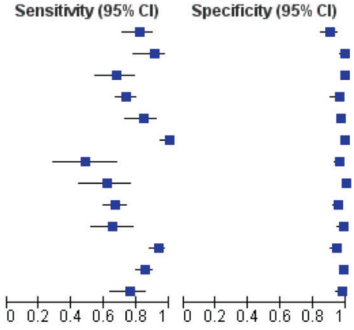
Standard 2

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]



TP true positive; FP false positive; FN false negative; TN true negative; CI confidence interval

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: Pooled sensitivity and specificity estimates.

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

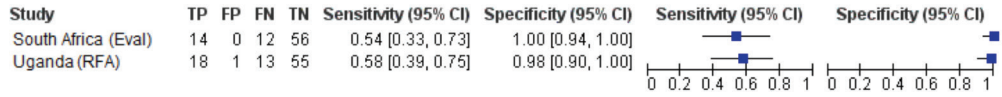
[1] All reference standards classify patients as having TB if ≥ 1positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients were required to have no positive and at least 1) two negative cultures on two different sputum specimens (Standard 1);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

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

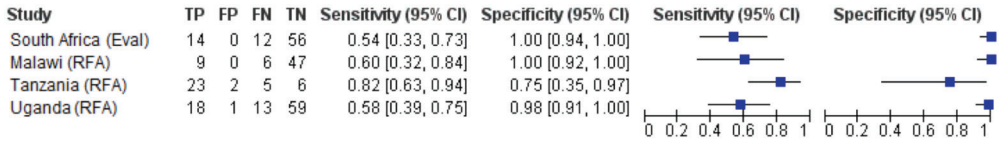
Most studies did not collect data on HIV status or most patients had unknown HIV status at the time of enrolment. Of 385 adults with known HIV infection eligible for inclusion in the analysis, 169 participants (44%) across 2 studies qualified for Standard 1, 271 participants (70%) across 4 studies qualified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

Figure 6: Forest plots of TB-LAMP sensitivity and specificity for the detection of TB among adults with HIV compared with three culture-based reference standards.

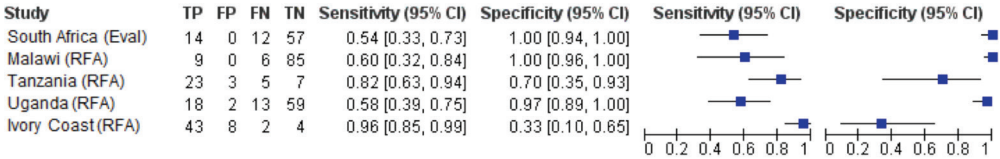
3A : Standard 1



3B : Standard 2



3C : Standard 3



TP true positive; FP false positive; FN false negative; TN true negative; CI confidence interval

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 indicates more heterogeneity in sensitivity estimates and specificity estimates with Standard 3 than with Standard 2 (insufficient studies 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).

Pooled sensitivity of TB-LAMP among HIV-infected adults was lower than among all adults, ranging from 63.8% (95% CI 49.0-76.4) with Standard 2 to 73.4 (95% CI 51.9-87.6) with Standard 3 (Table 5). Pooled specificity was low with Standard 3 (95.0%, 95% CI 64.0-99.5) but high with 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	Pooled sensitivity	Pooled specificity
Standard 1[1]	< 4 studies	< 4 studies
Standard 2[1]	63.8 (49.0-76.4)	98.8 (85.1-99.9)
Standard 3[1]	73.4 (51.9-87.6)	95.0 (64.0-99.5)

[1] All reference standards classify patients as having TB if ≥1positive 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

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 detection of smear-negative culture confirmed pulmonary TB was limited to adult participants with two negative sputum smear microscopy results. Of 2972 participants eligible for the analysis, 1349 (45%) across 7 studies qualified for Standard 1, 2190 (74%) across 9 studies for Standard 2, and 2916 (98%) across 11 studies for Standard 3 (Table 8).

Table 6. TB-LAMP as an add-on test following a negative sputum smear microscopy: Eligible and included patients according to reference standard and study site.

Study	Total	Eligible[2]	Included		
			Standard 1[1]	Standard 2[1]	Standard 3[1]
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	–	–	–
Tanzania (RFA)	648	489	–	438 (90%)	478 (98%)
Uganda (RFA)	233	149	149 (100%)	149 (100%)	149 (100%)
Ivory Coast (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 and 5 non-TB patients

[1] All reference standards classify patients as having TB if ≥1positive 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

[2] Total eligible includes missing and indeterminate LAMP results: Brazil EVAL (n=2 indeterminate), South Africa EVAL (n=2 indeterminate), Tanzania RFA (n=6 missing), Madagascar RFA (n=1 indeterminate).

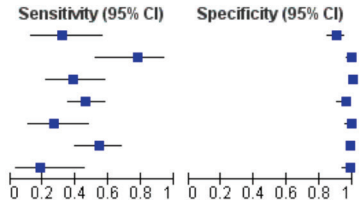
The sensitivity of TB-LAMP in individual studies ranged from 19-78% with Standard 1, 19-81% with Standard 2 and 17-81% with Standard 3 (Figure 7). There was significant heterogeneity in sensitivity estimates across studies, both from visual inspection of forest plots and statistical testing (I^2 74%-86%, $p < 0.001$ for all reference standards). As expected, 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).

The specificity of TB-LAMP in individual studies ranged from 90-100% with all standards (Figure 7). Visual inspection of forest plots indicated that heterogeneity in specificity estimates was less than for sensitivity estimates across studies, but was still significant (I^2 67-70.3%, $p < 0.005$ for all reference standards). 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) with Standard 3 to 98.4% (95% CI 95.9-99.4) with Standard 1 (Table 7).

Figure 7. Forest plots of TB-LAMP sensitivity and specificity for the detection of TB among adults as an add-on test following a negative sputum smear microscopy compared with three culture-based reference standards.

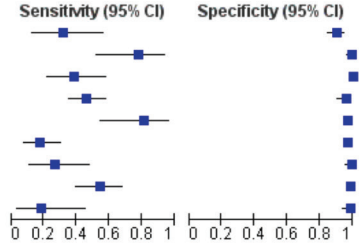
4A : Standard 1

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]



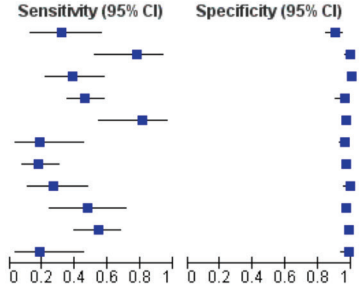
4B : Standard 2

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]



4C : 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]



TP true positive; FP false positive; FN false negative; TN true negative; CI confidence interval

The figure shows the estimated sensitivity and specificity of each study (square) and its 95% CI (horizontal line). The individual studies are ordered by decreasing sensitivity. 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	Pooled sensitivity	Pooled specificity
Standard 1[1]	42.1 (30.0-55.3)	98.4 (95.9-99.4)
Standard 2[1]	42.2 (27.9-57.9)	98.0 (96.0-99.0)
Standard 3[1]	40.3 (27.9-54.0)	97.7 (96.1-98.6)

[1] All reference standards classify patients as having TB if ≥1positive 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

5.4 Accuracy of TB-LAMP compared with Xpert MTB/RIF for detection of a pulmonary TB

The difference in diagnostic accuracy of TB-LAMP and Xpert MTB/RIF for the detection of pulmonary TB compared with a culture reference standard was determined for participants who underwent both TB-LAMP and Xpert MTB/RIF testing on non-frozen specimens. Of 2837 participants eligible for the analysis, 1075 (38%) across 4 studies qualified for Standard 1, 1809 (64%) across 6 studies for Standard 2, and 2772 (98%) across 8 studies for Standard 3 (Table 8).

Table 8. Diagnostic accuracy TB-LAMP compared with Xpert MTB/RIF: Eligible and included patients according to reference standard and study site.

Study	Total	Eligible[2]	Included		
			Standard 1[1]	Standard 2[1]	Standard 3[1]
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%)
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 and 5 non-TB patients
[1] All reference standards classify patients as having TB if at least one 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).
[2] Total eligible includes only patients with positive/negative TB-LAMP and Xpert MTB/RIF results

In this analysis, 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 pooled specificity from 98.2 (95% CI 96.0-99.2) to 98.9 (95% CI 97.4-99.6) across reference standards (Table 9). Pooled sensitivity of Xpert MTB/Rif ranged from 80.4% (95% CI 73.4-85.9) to 84.0% (95% CI 75.6-90.0) and pooled specificity 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 Xpert MTB/RIF: Pooled sensitivity and specificity.

Reference standard	Pooled sensitivity	Pooled specificity
TB-LAMP[1]		
Standard 1[2]	78.0 (66.6-86.4)	98.9 (97.4-99.6)
Standard 2[2]	74.1 (64.1-82.2)	98.8 (96.8-99.6)
Standard 3[2]	75.8 (63.2-85.0)	98.2 (96.0-99.2)
Xpert MTB/RIF[1]		
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)

[1] Data restricted to study participants who had valid results for both TB-LAMP and Xpert MTB/RIF and testing performed on non-frozen specimens.
[2] All reference standards classify patients as having TB if at least one 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 1) two negative cultures on two different sputum specimens (Standard 1); 2) two negative cultures on the same or different sputum specimens (Standard 2); or 3) at least one negative culture (Standard 3).

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

All 4 760 patients eligible for the analysis of TB-LAMP accuracy among all adults were included in the analysis determine the proportion of indeterminate/invalid TB-LAMP results (Table 3). The proportion of indeterminate LAMP results was 0% in 11 studies and 1% in two studies. There was minimal heterogeneity across studies (I2 28%, p=0.25). The pooled proportion of indeterminate TB-LAMP results was 0% (95% CI 0-0). Results were similar among HIV-infected adults; the pooled proportion of indeterminate TB-LAMP results in this sub-group 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 to the standard of care in settings where Xpert MTB/RIF testing coverage is limited[8].

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

Weighted average per-test cost of TB-LAMP was between $13.78-16.22 and $19.17-$28.34 for Xpert MTB/RIF when used as a routine diagnostic tests at all peripheral laboratories in both countries. First year expenditure required for implementation at medium workload peripheral laboratory (10-15 sputum smear microscopy tests per day) in Vietnam was $26,917 for TB-LAMP and $43,325.84 for Xpert MTB/RIF. These costs were approximately $3000 lower in Malawi, attributable to lower operating and staff costs. Likewise, TB-LAMP was considerably cheaper test to implement, accounting for 9.33% and 17.2% of the reported TB control budget for 2014 in each respective countries compared to 18% to 37% for Xpert MTB/RIF. In cost-effectiveness analysis, both of the TB-LAMP scenarios improved case detection rates and both strategies were cost-effective when comparing to the WHO willingness-to-pay threshold levels.

As a test performed at peripheral laboratories, TB-LAMP is generally cheaper and a more affordable molecular test alternative to Xpert MTB/RIF. 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 Xpert MTB/RIF cannot be implemented due to infrastructure requirements including continuous power supply. However, given the lack of capacity for TB-LAMP to detect rifampicin-resistant TB and sub-optimal sensitivity for the detection of TB among persons living with HIV, policy makers must cautiously evaluate operational feasibility and cost considerations prior to introducing this technology to their respective countries.

[8] H. Sohn, “Cost, affordability, and cost-effectiveness of TB-LAMP assay”. Report to WHO Guideline Development Group Meeting on TB-LAMP Assay, January 27th, 2016, Geneva.

7. Summary of evidence to recommendations

The accuracy of TB-LAMP performed directly on sputum samples for diagnosis of pulmonary TB among adults, not on TB treatment within 60 days of enrolment, was determined from 13 studies performed in intermediate and high-TB burden countries.

7.1. TB-LAMP for the detection of pulmonary TB in all adults

Using data from the 1,810 persons with signs and symptoms consistent with TB in whom the most stringent reference standard was available (Standard 1), TB-LAMP had a pooled sensitivity 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 that were misclassified as TB negative by the use of culture reference standard which varied across studies. All included studies were considered to have high risk of bias in the culture reference standard leading to misclassification of patients.

The GDG felt 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 7 more cases per 1000 individuals tested if the pre-test probability of TB is 5% and 22 more cases per 1000 individuals test if the pre-test probability of TB is 15% (Table 10). Correct identification of additional TB cases would be expected to lead to higher cure rates, less sequelae for the individual patient, and less transmission in the community.

The anticipated undesirable effect is the incorrect identification of an individual as a TB case when they are actually TB negative (FP). In this pooled data TB-LAMP had inferior performance to smear microscopy leading to an estimate of 16 more cases misclassified per 1000 individuals tested if the pre-test probability of TB is 5% and 14 more cases per 1000 individuals test if the pre-test probability of TB is 15% (Table 10). Incorrect identification of an individual as TB positive would lead to inappropriate treatment with potential medication toxicities to the individual, possible negative effect of stigmatization of the individual, negative economic effects for the individual and society. With a better reference standard, it can be expected that some false-positive TB-LAMP results would be re-classified as true positives, leading to improved sensitivity and specificity. Patients with non-tuberculous mycobacteria (NTM) 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 use of 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 (online annex 4).

7.2. TB-LAMP for the detection of pulmonary TB in adults with HIV

Data from 4 studies (271 participants) among HIV positive adults with signs and symptoms consistent with pulmonary TB evaluating the accuracy of TB-LAMP demonstrates sensitivity and specificity similar to sputum smear microscopy (64% and 62%) and (99% and 99%) respectively (Table 11). Based on this limited dataset very 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 the detection of TB using TB-LAMP in all patients with culture-confirmed TB and a 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-infected 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 the use of TB-LAMP in HIV positive persons but to reflect that there was limited evidence available (online annex 4).

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

Using data from the 1,349 persons with signs and symptoms consistent with TB across seven studies in which the most stringent reference standard was available (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% vs 100%), this may be partly explained by the identification of TB cases that were misclassified as TB negative by the use of culture reference standard which varied across studies. All included studies were considered to have high risk of bias in the culture reference standard leading to misclassification of patients.

The GDG felt 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 1000 individuals tested if the pre-test probability of TB is 5% and 63 more cases per 1000 individuals test if the pre-test probability of TB is 15% (Table 12). Correct identification of additional TB cases would be expected to lead to higher cure rates, less sequelae for the individual patient, and less transmission in the community.

The anticipated undesirable effect is the incorrect identification of an individual as a TB case when they are actually TB negative (FP). In this pooled data TB-LAMP had inferior performance to smear microscopy leading to an estimate of 19 more cases misclassified per 1000 individuals tested if the pre-test probability of TB is 5% and 17 more cases per 1000 individuals test if the pre-test probability of TB is 15% (Table 12). Incorrect identification of an individual as TB positive would lead to inappropriate treatment with potential medication toxicities to the individual, possible negative effect of stigmatization of the individual, negative economic effects for the individual and society. With a better reference standard, it can be expected that some false-positive TB-LAMP results would be re-classified as true positives, leading to improved sensitivity and specificity. Thus the GDG felt that it would be expected that use of TB-LAMP as an add-on test following a negative for 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 Xpert MTB/RIF for the detection of pulmonary TB in adults

Although better than microscopy, the pooled sensitivity of TB-LAMP is lower than what has been reported for Xpert MTB/RIF (89%, 95% CI 85-92)[9]. The specificity of all three tests is similar. In head-to-head comparisons, TB-LAMP appeared to be less sensitive than Xpert MTB/RIF, but the sensitivity difference was not statistically significant except when using the least stringent reference standard. More data is needed to confirm that TB-LAMP sensitivity is lower than that of Xpert MTB/RIF, it is also important to consider that TB-LAMP has a different end-user profile.

The GDG felt that TB-LAMP could be implemented as a replacement test for microscopy in peripheral microscopy centers where the laboratory infrastructure and requirements for continuous

[9] Steingart KR, Schiller I, Horne D, Pai M, Boehme CC, Dendukuri N. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Cochrane Database of Systematic Reviews. 2014;(1).

power supply restrict the implementation of Xpert MTB/RIF. The evidence to date along with increased automation, fewer training requirements, and the ability to identify rifampin resistance suggest that Xpert MTB/RIF should remain the preferred diagnostic whenever there are sufficient resources and infrastructure to support its use. The GDG acknowledged the importance of reaching the targets in the End TB Strategy prioritizing implementation of diagnostics that allow for an early diagnosis of tuberculosis 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 that:

1. TB-LAMP may be used as a replacement test for sputum smear microscopy for the diagnosis of pulmonary TB in adults with signs and symptoms consistent with TB (Conditional recommendation, Very low quality of 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 of evidence).

Remarks

- These recommendations apply to settings where conventional sputum smear microscopy is able to 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 MDR-TB;
- The test has limited additional diagnostic value over sputum smear microscopy for the testing of persons living with HIV with 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 the use of the TB-LAMP assay in children, based on the generalization of data in adults, while acknowledging difficulties in the collection of sputum specimens from children.

9. Implementation considerations

The systematic review supports the use of TB-LAMP as a replacement test for smear microscopy for diagnosis of pulmonary TB in intermediate and high TB burden countries. The evidence to date along with increased automation and the ability to simultaneously identify rifampin resistance suggest that Xpert MTB/RIF should remain the preferred diagnostic for all persons suspected of having TB providing there are sufficient resources and infrastructure to support its use.

- Several operational issues would need to accompany an implementation of the TB-LAMP assay: the need for electricity supply, adequate storage and waste disposal, stock monitoring, and temperature control in storage settings where temperatures are above manufacturer's recommendation (currently 30°C for TB-LAMP);
- TB-LAMP is designed and evaluated to detect ***M. tuberculosis*** in sputum specimens. Other samples (e.g. urine, serum, plasma, CSF or other body fluids) have not been adequately evaluated;
- TB-LAMP should be used in the direct testing of sputum samples irrespective of whether samples are smear-negative or smear-positive;
- Adoption of the TB-LAMP assay does not eliminate the need for smear microscopy, which should be used for monitoring of treatment of drug susceptible TB patients. However, the demand for conventional sputum microscopy may decrease in settings where TB-LAMP may fully or partially replace conventional sputum microscopy;
- TB-LAMP assay should not replace Xpert MTB/RIF for the following reasons: ability of the latter to simultaneously detect MTB and rifampicin resistance, automation and relative simplicity of the procedure;
- In settings, where Xpert MTB/RIF cannot be implemented (inadequate electric supply, temperature, humidity, excessive dust), TB-LAMP may become a plausible alternative to the latter.

10. Plans for disseminating the WHO policy guidance on TB-LAMP

This WHO policy guidance will be published online (http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/) and disseminated through WHO/GTB Department listserve to all WHO Regional and Country Offices, Member States, the Global Laboratory Initiative and New Diagnostics Working Groups of Stop TB Partnership, donors, technical agencies and other stakeholders.

11. Research needs

The current recommendations on the commercial TB-LAMP 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 Xpert MTB/RIF. Further operational research on the TB-LAMP test should focus on the following priorities:

- Evaluation of diagnostic algorithms in different epidemiological and geographical settings and patient populations;
- Conducting more rigorous studies with higher quality reference standards (including multiple specimen types, also extrapulmonary) to improve confidence in specificity estimates;
- Determine training, competency, and quality assessment needs;
- Gathering more evidence on the impact on TB treatment initiation and mortality;
- Perform country-specific cost-effectiveness and cost-benefit analyses of targeted TB-LAMP use in different programmatic settings;
- Meet “Standards for reporting Diagnostic Accuracy studies” (STARD) for future studies.

12. Annexes

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

References to studies included in the review

1. Hang PT, Peter J, Mello FCQ, Parraga T, Nguyen TNL, Nabeta P. et all. Performance of the TB-LAMP Diagnostic Assay in Reference Laboratories – Results from a Multi-Center Study (Brazil, Peru, South Africa, Vietnam). Submitted for publication to International Journal of Tuberculosis and Lung Diseases, April 2016.
2. Gray CM, Katamba A, Narang P, Giraldo J, Zamudio C, Joloba M et all. Feasibility and operational performance of TB LAMP in decentralized settings – results from a multi-center study. Submitted for publication to Journal of Clinical Microbiology, April 2016.
3. Study conducted under FIND LAMP RFA MOU - India. Not published.
4. N'guessan K, Horo K, Coulibaly I, Adegbele J, Kouame-Adjei N, Seck-Angu H et all. Comparison of pulmonary tuberculosis diagnosis by AFB detection, TB-LAMP and GeneXpert MTB/RIF in Ivory Coast. (Unpublished study - Abstract see Annex 3).
5. Study conducted under FIND LAMP RFA MOU - Madagascar. Not published.
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 for publication to PLOS One, March 2016.
7. Khalief MS, Doulla B, Mtunga DD, Adepoyibi T, Moriera R, Boyle DS. Evaluation of a loop-mediated isothermal amplification test kit for the diagnosis of pulmonary tuberculosis in the United Republic of Tanzania. (Unpublished study - Abstract see Annex 3).
8. Study conducted under FIND LAMP RFA MOU – Uganda. Not published.
9. Study conducted under FIND LAMP RFA MOU - Vietnam. (Unpublished study - Abstract see Annex 3).
10. Kaku T, Minamoto F, D'Meza R, Morose W, Boncy J, Bijou J, Geffrard H, Yoshida M, Mori T. Assessment of Accuracy of LAMP-TB Method for Diagnosing Tuberculosis in Haiti. Japanese Journal of Infectious Diseases, March 2016.

References to studies excluded from this review

1. Study conducted under FIND LAMP RFA MOU - Cameroon. Not published.
2. Study conducted under FIND LAMP RFA MOU – Cambodia. Not published.
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. Not published.
4. Bojang AL, FS Mend , LD Tientcheu, J Otu, M Antonio, B Kampmann , S Agbla , JS Sutherland. Comparison of TB-LAMP, GeneXpert MTB/RIF and culture for diagnosis of pulmonary tuberculosis in The Gambia. J Infect. March 2016; 72 (3): 332-7.
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6. Study conducted under FIND LAMP RFA MOU - Mongolia. Not published.
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Annex 2. GRADE Evidence Profiles

Table 10. GRADE evidence profile: Accuracy of TB-LAMP as a replacement test for sputum smear microscopy for the diagnosis of pulmonary tuberculosis 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 studies

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)

Prevalences	5%	15%	

Outcome	N° of studies (N° 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 tuberculosis)	7 studies 1 810 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	very serious[3]	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 tuberculosis)								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 tuberculosis)	7 studies 1 810 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	serious[4]	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 tuberculosis)								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**		

TN - true negative; TP - true positive; FN - false negative; FP - false positive; QoE - quality of evidence
1. The QUADAS-2 tool was used to asses the risk of bias. One study performed only LJ culture (Madagascar RFA); 6 studies that performed MGIT liquid culture but had culture contamination rate less than 5%, 2 studies (Uganda RFA, Haiti study) did not exclude all participants with prior TB, 3 studies (Madagascar RFA, Uganda RFA, Haiti study) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
2. There were serious concerns for applicability as no studies were conducted in peripheral microscopy centers (4 were performed at reference laboratories and 3 were performed at hospital-/university-affiliated outpatient clinics). The evidence was downgraded by one point.
3. There was considerable heterogeneity in sensitivity estimates across individual studies. The evidence was downgraded by one point.
4. 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 the diagnosis of pulmonary tuberculosis among all adults 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 studies

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)

Prevalences	5%	15%	

Outcome	N° of studies (N° 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 tuberculosis)	4 studies 271 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	not serious	serious[3]	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 tuberculosis)								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 tuberculosis)	4 studies 271 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	not serious	serious[3]	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 tuberculosis)								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**		

TN - true negative; TP - true positive; FN - false negative; FP - false positive; QoE - quality of evidence
1. The QUADAS-2 tool was used to asses the risk of bias. There were insufficient studies to obtain pooled estimates using reference standard 1; 2 studies that performed MGIT liquid culture had culture contamination rate less than 5% (South Africa EVAL, Uganda RFA); 2 studies (Tanzania RFA, Uganda RFA) did not exclude participants with prior TB; 1 study (Malawi RFA) had less than 20% of eligible participants excluded because of insufficient culture data for reference standard 2; and 2 studies (Tanzania RFA, Uganda RFA) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
2. There were serious concerns for applicability as only one study was conducted in a peripheral microscopy center. One study was performed in a reference laboratory and two studies were performed in hospital or university affiliated outpatient clinics). The evidence was downgraded by one point.
3. Small sample size and wide confidence intervals for pooled estimates. The evidence was downgraded one point for imprecision.

Table 12. GRADE evidence profile: Accuracy of TB-LAMP as an add-on test following a negative sputum smear microscopy to diagnose pulmonary tuberculosis 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 studies

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

Prevalences	5%	15%	

Outcome	N° of studies (N° 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 tuberculosis)	7 studies 1 349 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	very serious[3]	not serious	none	21 (15 to 28)	63 (45 to 83)	**VERY LOW**
False negatives (patients incorrectly classified as not having pulmonary tuberculosis)								29 (22 to 35)	87 (67 to 105)	
True negatives (patients without pulmonary tuberculosis)	7 studies 1 349 patients	Cross-sectional (cohort type accuracy study)	very serious[1]	serious[2]	serious[4]	not serious	none	931 (912 to 941)	833 (816 to 842)	**VERY LOW**
False positives (patients incorrectly classified as having pulmonary tuberculosis)								19 (9 to 38)	17 (8 to 34)	

TN - true negative; TP - true positive; FN - false negative; FP - false positive; QoE - quality of evidence
1. The QUADAS-2 tool was used to asses the risk of bias. One study performed only LJ culture (Madagascar RFA); 6 studies that performed MGIT liquid culture but had culture contamination rate less than 5%, 2 studies (Uganda RFA, Haiti study) did not exclude all participants with prior TB, 3 studies (Madagascar RFA, Uganda RFA, Haiti study) did not clearly report the number of patients enrolled. The evidence was downgraded by two points.
2. There were serious concerns for applicability as no studies were conducted in peripheral microscopy centers (4 were performed at reference laboratories and 3 were performed at hospital-/university-affiliated outpatient clinics). The evidence was downgraded by one point.
3. There was considerable heterogeneity in sensitivity estimates across individual studies. The evidence was downgraded by one point.
4. 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

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Background: The specificity of acid-fast microscopy is excellent for mycobacterial species, but its sensitivity is not optimal. Unfortunately, its remains the main tool for pulmonary tuberculosis diagnosis in low-incomes countries. Early diagnosis and an appropriated treatment are important for tuberculosis control. This study aim was to determine performances of AFB detection after Ziehl-Neelsen staining, Loop-Mediated Amplification test for (TB-LAMP) and GeneXpert MTB/RIF assay for tuberculosis diagnosis to symptomatic patients versus culture in liquid medium.

Methods: Sputum samples of patients recruited at CAT de Yopougon (intermediate laboratory of tuberculosis) were collected. On the same sputum, direct examination after Ziehl-Neelsen staining and TB-LAMP were respectively blindly performed. Samples were then transported at Institut Pasteur de Côte d'Ivoire in Ice box at 4°C and decontaminated according to NALC method. After centrifugation pellet was used for MGIT culture and 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%) Comparatively to culture, sensitivity and specificity of Direct examination were respectively 86% (95% IC : 81-91) 96% (95% IC : 94-98). Sensitivity of GeneXpert MTB/RIF assay was 96% (95% IC : 0.93-0.99) and 92% (95% IC: 0.88-0.96) for TB-LAMP. Specificity of molecular method was 90% (95% IC: 87-93) and 94% (95% IC: 91-97) respectively. In total, 147 (31.3%), 162 (34.5%), 183 (39%) active pulmonary TB cases were detected respectively with smeart examination, TB-LAMP and GeneXpert TB/RIF.

Conclusion: Compare to microscopy, molecular methods increased TB cases diagnosed of 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

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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 with presumptive pulmonary TB over 18 years of age were tested for TB with LAMP, SSM and Xpert MTB/RIF. After data review, a sample set of 550 participants were used. As compared to culture, the sensitivity and specificity of the Loopamp assay were 67.4% and 97.5% respectively. 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 result, the the sensitivity and specificity 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 shows that while the Loopamp assay has greater sensitivity when compared to 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

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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 potential to replace the conventional smear microscopy test. This study aimed to evaluate the LoopampTMMTBC detection kit, that uses the Loop-mediated isothermal amplification for TB (TB-LAMP), at a peripheral laboratory in Vietnam.

From January 2014 to April 2014, 503 people with TB typical symptoms were consecutively enrolled from Ung Hoa district, Hanoi, Vietnam. Three sputum samples (two spots and one morning) were collected from each of these TB presumptive cases. Single TB-LAMP test done at the district health center laboratory was compared with three-smear microscopy and single GeneXpert MTB/RIF using single culture as the reference test. Test results were available from 498 subjects with mean age ± SD of 58.6 years ± 14.9, 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%) and 15.8% (95%CI 33.8% - 39.6%) in smear-positive/culture-positive and smear-negative/culture-positive samples, respectively. 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 Xpert/RIF assay and smear microscopy. Whilst Xpert/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, respectively). Although the TB-LAMP specificity was lower than that of smear microscopy (95.1% vs. 98.9%), the single TB-LAMP test has sensitivity equal to that of three-smear microscopy and higher than any single smear microscopy. The LoopampTMMTBC detection kit, therefore, could be possible for replacing the AFB smear microscopy at peripheral laboratories of high TB prevalence areas. However, the kit should be improved on technology, design and implementing procedure in order to increase its sensitivity and specificity when being applied at this primary level.

THE
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