THE USE OF A COMMERCIAL LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY (TB-LAMP) FOR THE DETECTION OF TUBERCULOSIS

EXPERT GROUP MEETING REPORT
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Executive summary

Background

Nucleic acid amplification offers major advantages of speed and sensitivity for pathogen detection, but until recently, no commercial versions of these systems were designed to be simple enough or inexpensive enough to implement in resource-limited countries. Recent research and development efforts have, however, led to the development of new molecular approaches which may change this paradigm.

TB-LAMP is a new manual TB detection method based on the novel loop-mediated isothermal amplification (LAMP) platform from Eiken Chemical Co. in Japan. TB-LAMP has several features that makes it attractive as a diagnostics platform for resource-poor settings: it is fast (15-40 min), isothermal (requiring only a heat block), robust to inhibitors and reaction conditions that usually adversely affect polymerase chain reaction (PCR) methods, and it generates a result that can be detected with the naked eye. Since 2005, FIND and Eiken have been collaborating to develop an assay for TB that could be implemented in place of microscopy to improve the accuracy of TB detection at microscopy centres and similar laboratories.

The agreed performance targets for the development efforts were such that the new test must not only be equal or superior to sputum smear microscopy in ease-of-use, speed, and specificity, but that it must also be significantly more sensitive. During development, a number of important design changes were made to try to meet this goal, including the development of a simple manual extraction technology. Assay development is now completed, and a TB LAMP kit is registered with the Japanese regulatory authorities and CE marked.

The TB LAMP assay is designed to require, as much as possible, a similar number of steps and biosafety requirements as sputum smear microscopy. Users pipette-transfer a small volume of sputum to a heating tube already containing lysis mix and heat this at 90°C for 5 minutes. The heating tube is then joined to a tube containing an absorbent material which removes amplification inhibitors. The extract can then be expressed directly from this tube into the reaction tube, which contains dried-down reagents under the cap. After inversion to reconstitute the reaction mix, tubes are placed into the warming block for 40 minutes and the stable result, which is turbid and fluorescent, is examined with the naked eye. There are no moving parts to the system, and no requirement for additional pieces of equipment or reagents.

Formal evaluation in a hospital setting in Japan was carried out to support Japanese registration. Subsequently, a series of clinical studies were carried out by FIND in reference centres and in settings of intended use to determine the performance and applicability of the assay relative to microscopy and with conventional culture as reference standard in high-burden TB countries.

Summary of results

Validation study (industry sponsored): This study involved 170 patients at two hospital settings in Japan. 320 TB-LAMP tests were performed of which 205 were positive. The sensitivity of TB-LAMP was 98.2% among smear-positive/culture-positive samples and 55.6% among smear-negative/culture-positives. Specificity for TB diagnosis was 93.9%.

Evaluation studies in TB reference laboratories: Multi-centre evaluation studies involving 1061 patients tested in reference laboratories in Vietnam, South Africa, Peru and Brazil showed that TB-LAMP detected almost 97% of smear-positive/culture-positive patients and 53% of smear-negative/culture-positive patients. Indeterminate rates were very low (<0.2%); however DNA contamination events were observed in some testing runs. The specificity of the TB-LAMP test across these studies was 94.7%, below the original performance target of 97%.

Users in TB reference laboratories found the operational aspects of TB LAMP generally advantageous. However, the failure of specificity meeting the original performance targets, and the lack of evidence that DNA contamination was responsible, led to a root cause analysis of false-positive results. Exposure of reaction tubes to humidity was identified as one of the possible causes of false-positives. Consequently, changes in packaging were made to improve the resealing of aluminum pouches containing the reaction tubes.

Evaluation studies in settings of intended use: Subsequent studies were carried out in 11 rural or simple urban microscopy centres in India, Uganda and Peru that were representative of settings of intended use and similar to those where sputum smear microscopy is the available routine diagnostic option. These settings had limited

bench space, frequent power outages, uncontrolled and often high ambient temperatures, and had no staff with prior molecular training.

Staff were trained intensively with standardised materials and underwent proficiency testing before study initiation. 1741 patients met the enrolment criteria and had a clear final diagnosis using local microscopy, with liquid plus solid media culture conducted as reference standard in a supervisory reference laboratory. All TB-LAMP positive but culture-negative results were considered to be false-positive.

In these settings of intended use, TB-LAMP performance was slightly superior to earlier studies in reference laboratories, detecting 97% of smear-positive patients and 62% of smear-negative TB. Indeterminate rates (1.5%) were slightly higher than in the studies in reference settings. The specificity of TB-LAMP (96.3%) was lower than that of microscopy (97.3%). The occurrence of false-positive results was not uniformly distributed across sites - specificity of TB-LAMP in Uganda and Peru was around 97%, but was 94% in India.

The root cause analysis showed that in settings of high heat and humidity, such as experienced in India during the studies, failure to follow manufacturer's instructions, especially with regard to delays in either reconstituting the dried reagents or in starting the amplification reaction after reconstitution, could cause non-specific amplification in the absence of target *M. tuberculosis* DNA. This risk was exacerbated by the addition of inadequate volume of extracted DNA in the reaction tube. As a result of these findings, small alterations were made in the assay. The recommended reaction volume was increased from 25-35 µl to 30-35 µl, and training was altered to stress the importance of following procedural recommendations to avoid false-positive results.

Repeat evaluation studies in settings of intended use (Indian sites only): To assess the effectiveness of these modifications, a repeat enrollment was carried out in the same settings in India with additional training focusing on the temperature-sensitive steps identified during the root-cause analysis. 417 patients meeting study criteria were enrolled. No further evaluation of the revised protocol was undertaken. Overall, TB-LAMP specificity increased to 97.6% (95%CI 95.5-98.7). However, there were variations in the number of specimens tested and the specificity at the three different test sites varied. Almost 50% of the samples were tested at one site with a TB-LAMP specificity of 96.2% (95%CI 92.4-98.1).

End-user feedback: Operational appraisals were gathered from end users (laboratory technicians) and experts (laboratory directors, physicians and microbiologists) through questionnaire surveys. Experienced users generally found TB-LAMP to be simpler than microscopy, and preferable in settings with a microscopy workload exceeding 20 samples per day. Overall feedback from experienced users was positive and almost all agreed that TB-LAMP could be implemented at routine laboratories, and that it was less complex and faster than smear microscopy. However, the same users also stressed the possible risks for cross contamination, false-positive results, the user-dependence of TB-LAMP results, and the need for comprehensive training and quality assurance, which together with cost were seen as the most important obstacles to widespread implementation.

Expert Group Findings

The Expert Group recognized that TB-LAMP is a new assay which offers a manual molecular approach to TB detection that seems to be feasible in peripheral laboratories following extensive training. Several operational issues which would need to accompany any such technology were regarded as relevant: 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 has the advantages of being relatively high-throughput, not requiring sophisticated instrumentation, and being self-contained, without the need for complex biosafety facilities or ancillary equipment. The Expert Group did, however, note that these benefits must be weighed against the need for extensive training and quality assurance required to achieve reproducible results and the anticipated test cost relative to sputum smear microscopy.

Considering the balance of benefits and harms associated with implementing the TB-LAMP assay, the Expert Group noted that, in settings with a TB prevalence of 5%, the average positive predictive value of the test was 51.2% across the studies (range 33.3% - 56.6%). In such settings the number of true-positive results would be almost the same as the number of false-positive results. The Expert Group also noted that changes to the assay made following the detection of false positive-results in the India study was only re-evaluated in the one site in India where the false positive-results were initially observed.

The Expert Group was concerned that the modified assay was not re-evaluated at other evaluation settings of intended use, including high HIV prevalence settings. Furthermore, the Expert Group raised concerns that conventional Ziehl-Neelsen microscopy was used as the comparison test with the TB-LAMP assay and recommended that comparison should be made with LED fluorescence microscopy, given that LED microscopy is now regarded as the reference standard.[1] The Expert Group also noted that batching of samples (up to 14 samples) would minimize the cost per test given that the manufacturer recommends for a positive and negative control test to be included in each batch of the TB LAMP assay. However, concerns were raised that batching tests to minimize test costs could result in diagnostic delays for patients.

Expert Group Recommendations

The Expert Group agreed that LAMP technology has potential as a rapid TB diagnostic tool but that the body of evidence presented on the TB-LAMP assay was insufficient to make a recommendation either in favour of, or against the use of TB-LAMP as a replacement test for sputum smear microscopy. The Expert Group made the following recommendations to improve the evidence base for TB-LAMP:

- The specificity of the TB-LAMP assay remains a major concern especially when the TB prevalence falls below 10%. In these settings (often found in high-burden TB countries), the positive predictive value for the assay is insufficient. Further research is therefore needed to improved assay specificity, especially for high-burden TB settings;

- Further studies in different geographical regions are needed, especially in high HIV prevalence settings where the sensitivity of sputum smear microscopy is reduced;

- Head-to-head comparison studies with TB-LAMP and LED microscopy are recommended given the increased sensitivity of LED compared with conventional light microscopy[1];

- Further research is recommended to simplify the technology and increase the user robustness of the assay, especially in settings where staff are unfamiliar with manual molecular techniques;

- The anticipated cost of the TB-LAMP assay relative to microscopy was perceived as a major barrier to implementation and scale-up;

- Evaluation of the TB-LAMP assay by more investigators is encouraged to enable further independent assessment.

[1]World Health Organization. *Policy Statement on LED Fluorescence Microscopy*. World Health Organization: Geneva, 2011. Available at: http://www.stoptb.org/wg/gli/documents.asp

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