6. SUMMARY

- The present cross-sectional analytical study was aimed at developing a new \textit{mpt64} based LAMP assay and study its analytical sensitivity and specificity as well as to evaluate it on sputum samples from pulmonary TB suspects. The performance of this assay was compared with other molecular tests i.e. LAMP (Markers: IS6110) and PCR (Markers: IS6110 and \textit{mpt64}).
- A new set of LAMP primers targeting \textit{mpt64} antigenic protein were designed using the Primer Explorer v4 software on the Eiken Chemical Ltd. (Japan) website. Corresponding loop primers were generated.
- The temperature gradient varying from 61-65°C and betaine concentrations (0.6M, 0.8M, 1M and 2M) were standardized for the optimal performance of the LAMP assay.
- The results of the LAMP assay could be observed visually by adding 10µl of (100xdiluted) SYBR Green I dye.
- Analytical sensitivity of the assay was checked by setting up a gradient of \textit{M. tuberculosis} H37Rv DNA dilutions (10^{-10} \text{ng/ml} – 10\text{ng/ml} ) and was found to be 1pg/ml.
- Analytical specificity of the assay was assessed by setting up the LAMP reaction with 1ng DNA from different mycobacterial and non-mycobacterial species. A positive LAMP assay was observed only with members of the \textit{Mycobacterium tuberculosis} complex.
- 230 patients suspected of pulmonary TB were enrolled in which a diagnosis of pulmonary TB was made based on bacteriological or clinical confirmation of TB, which included clinical judgement, radiological evidence and microbiological positivity.
- Of the 230 patients, 7 were excluded because of culture contamination. Out of the remaining 223 patients, 119 were classified as PTB positive on the basis of bacteriological or clinical evidence and 104 as non-TB control cases. Smear microscopy, solid LJ culture, LAMP and PCR targeting DNA sequences of \textit{mpt64} and IS6110 were done on all samples of the patients.
- Among all the tests used for diagnosis, \textit{mpt64} LAMP exhibited the highest sensitivity (100%) followed by IS6110 LAMP (93.5%), then \textit{mpt64} PCR (84.0%) and IS6110 PCR (81.8%).
- \textit{mpt64} PCR showed the highest specificity (100%) followed by IS6110 PCR (98.0%), IS6110 LAMP (97.1%) and \textit{mpt64} LAMP (96.1%).
- Similarly, \textit{mpt64} PCR had the highest PPV (100%) followed by IS6110 PCR(97.9%), IS6110 LAMP(97.3%) and \textit{mpt64} LAMP (96.75%).
- \textit{mpt64} LAMP had the highest NPV (100%) followed by IS6110 LAMP (92.6%), \textit{mpt64} PCR (84.5%) and IS6110 PCR(82.2%).
Summary

- mpt64 LAMP had the maximum accuracy when compared with all the tests with an area under the curve (AUC) in the receiver operating characteristic (ROC) of 0.981 and IS6110 LAMP had an AUC of 0.952 followed by mpt64 PCR (0.920) and IS6110 PCR(0.898).
- In the S⁺ C⁺ group, mpt64 LAMP and IS6110 LAMP both had a sensitivity of 100%, followed by mpt64 PCR (93.3%) and IS6110 PCR(93.2%).
- In the S⁻ C⁺ PTB+ group, there were 15 samples all of which were detected by mpt64 LAMP, followed by IS6110 LAMP (11/15), mpt64 PCR (11/15) and IS6110 PCR(10/15). In the S⁺ C⁻, PULMONARY TB group, again mpt64 LAMP detected all the 8 samples whereas IS6110 LAMP, mpt64 PCR and IS6110 PCR detected 7, 5 and 4 samples each respectively.
- In smear negative TB patients diagnosed by clinical and bacteriological methods, mpt64 LAMP had a sensitivity of 100% followed by IS6110 LAMP (85.7%), mpt64 PCR (52.3%) and IS6110 PCR (47.6%).
- In the culture negative PTB patients diagnosed by clinical and biological criteria, mpt64 LAMP had a sensitivity of 100% followed by IS6110 LAMP (71.4%), mpt64 PCR (35.7%) and IS6110 PCR (28.5%).
- In bacteriologically negative (S⁻ C⁻) patients, mpt64LAMP had a sensitivity of 100% whereas IS6110 LAMP had a sensitivity of 50%. mpt64 PCR and IS6110 PCR failed to detect any of these samples.
- In bacteriologically positive patients, mpt64 LAMP had a sensitivity of 100% followed by IS6110 LAMP (95.5%), mpt64 PCR (88.5%) and IS6110 PCR (85.8%).
- LAMP had a higher analytical sensitivity than PCR, especially in bacteriologically negative samples, which is due to the large amount of DNA produced during the amplification process and should fare better than PCR based Xpert-MTB/RIF in patient care.
- Based on the performance of molecular tests in the present study it can be suggested that NAAT should always be used in conjunction with smear or culture tests to increase the sensitivity of diagnosis.
- The best combination of techniques for TB diagnosis would be LAMP and mycobacterial culture. LAMP method has high sensitivity in paucibacillary TB infections which are difficult to diagnose due to low bacterial load. It fills up the gap caused by low culture positivity of paucibacillary TB infections. Alternatively, mycobacterial culture increases the specificity of the combination.
- From the point of view of patient care, combining LAMP with a simple procedure like smear microscopy is a good option. Both the tests are simple, rapid and feasible at low cost even in simple laboratory set-ups.