Proceedings of the 6th International Conference on Theoretical and Applied Nanoscience and Nanotechnology (TANN'22)
Niagara Falls, Canada – June 02-04, 2022
Paper No. 121
DOI: 10.11159/tann22.121

Solid Phase Reversible Immobilization (SPRI) Beads-Based Detection of *Mycobacterium Leprae* Positive Samples Using Isothermal Technique

Nupur Garg[1], Farhan Jalees Ahmad[1], Sudeshna Kar[2]
[1]Nanomedicine Research Lab, School of Pharmaceutical Education and Research, Jamia Hamdard
NewDelhi, India
nupursgarg_sch@jamiahamdard.ac.in; fjahmad@jamiahamdard.ac.in
[2]Department of Molecular Medicine, Jamia Hamdard
New Delhi, India
sudeshna.kar@jamiahamdard.ac.in

Extended Abstract

The cases of leprosy, caused by *Mycobacterium leprae* (ML), are still stagnant owing to the ongoing transmission and neglection. [1] Cases mostly present in poor resource-setting areas where diagnosis is done by bacilloscopy, lab tests or PCR methods limited to healthcare infrastructure.[2] ML has vast genomic similarity with *M. tuberculosis* due to which cases of misdiagnosis occur. Since, there has been a re-emerging situation of leprosy owing to the perpetuating transmission in endemic areas, a specific, sensitive, rapid and economical diagnostic method with minimum field settings is obligatory. Loop-mediated isothermal amplification (LAMP) DNA amplification method offers a single-tube-single-temperature simple amplification method using minimum equipment, easy-to-operate, portable, affordable and an ideal tool for field settings that lack resources. [3] SPRI beads are paramagnetic nanobeads consisting polystyrene core enveloped by magnetite and carboxylate coating that can bind with long amplified DNA molecules under magnetic field. Amplified DNA forms bridges between beads forming floccules. These floccules in form of precipitates could be visualized by naked eyes providing a visual identification method of presence of target DNA called Bridge Flocculation Assay.[4]

Optimization of LAMP method for *M. leprae* gene sequence amplification was done using three sets of primers with reaction reagents for reaction parameters showing maximum amplification in 60 min at constant temperature of 66ºC. Conventionally, detection of amplicons is done by gel electrophoresis which is qualitative and tedious; or fluorescent dyes that cause non-specific binding. We coupled LAMP assay with SPRI detection of mycobacterial presence in samples. After amplification, column purification of amplicons was done to remove salts and primers, and incubated with 1.5 times SPRI beads solution in a magnetic separator. [5]

Under magnetic field, the nanobeads bind with amplified DNA and form a visible pellet in contrast to a dispersion in the absence of target DNA. The sensitivity of method was evaluated using serial dilutions and the limit of detection was found to be in picograms. The selectivity was evaluated using *M. tuberculosis* as negative control and found to be 100% implying the method is specific for *M.leprae* detection. Hence, LAMP-SPRI assay displays the potential to be a rapid, portable, simple and affordable diagnostic tool for leprosy that could be used in endemic areas having limited healthcare resources.

References

[1] https://www.who.int/data/gho/data/indicators/indicator-details/GHO/number-of-new-leprosy-cases. (Updated 11.01.2022).
[2] U. Sengupta, "Recent laboratory advances in diagnostics and monitoring response to treatment in leprosy," *Indian Dermatol. Online J.*, vol. 10, no. 2, p. 106, Mar. 2019, doi: 10.4103/idoj.IDOJ_260_18.
[3] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, & T. Hase. (2000). Loop-mediated isothermal amplification of DNA. Nucleic acids research, 28(12), E63. https://doi.org/10.1093/nar/28.12.e63., "Loop-mediated isothermal amplification of DNA," *Nucleic Acids Res.*, vol. 28, no. 12, p. e63, Jun. 2000.

[4] E. J. H. Wee, H. Y. Lau, J. R. Botella, and M. Trau, “Re-purposing bridging flocculation for on-site, rapid, qualitative DNA detection in resource-poor settings,” *Chem. Commun. Camb. Engl.*, vol. 51, no. 27, pp. 5828–5831, Apr. 2015, doi: 10.1039/c4cc10068a.

[5] N. Garg, U. Sahu, S. Kar, and F. J. Ahmad, “Development of a Loop-mediated isothermal amplification (LAMP) technique for specific and early detection of Mycobacterium leprae in clinical samples,” *Sci. Rep.*, vol. 11, no. 1, Art. no. 1, May 2021, doi: 10.1038/s41598-021-89304-2.