RAPID DETECTION OF *Mycobacterium* IN SPUTUM SAMPLES IN NEPAL BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

A
DISSERTATION
SUBMITTED TO THE CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY
(ENVIRONMENTAL AND PUBLIC HEALTH)

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2011

RECOMMENDATION

This is to certify that Miss Manju Tamang has completed this dissertation work entitled " **Rapid Detection of *Mycobacterium* in sputum samples in Nepal by Loop-Mediated Isothermal Amplification (LAMP)** " as a partial fulfillment of **Master of Science Degree in Microbiology** under our supervision. To our knowledge, this work has not been submitted for any other degree.

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ACKNOWLEDGEMENTS

First of all, I would like to express my sincere and great debt of gratitude to my respected supervisor Dr. Dwij Raj Bhatta, Head, Central Department of Microbiology, Tribhuvan University, Kirtipur for his inspiration, full support and expert guidance in the completion of thesis.

I would like to express my deep sense of gratitude and heartfelt appreciation to my respected supervisors Dr. Basu Dev Pandey, Consultant Physician, Sukraraj Tropical and Infectious Disease Hospital, Teku and Director of Everest International Clinic and Research Center (EICRC) for laboratory facility, his supervision, motivation, technical support that I received during entire research period and Mr. Binod Lekhak, Assistant Professor Central Department of Microbiology, Tribhuvan University, for his constant inspiration and valuable suggestions during the entire period of my dissertation work.

I am thankful and much obliged to all the respected teachers and staffs of Central Department of Microbiology for their full fledge support.

I am very much grateful to Dr. Bhawana Shrestha, Project Chief, German-Nepal Tuberculosis Project (GENETUP), and Mr. Bhagawan Maharjan, laboratory incharge (GENETUP), for their help during sample collection and over all laboratory performance. I am thankful to Mr. Suraj Shrestha, Mr. Sujit Maharjan, Mrs. Meera Shrestha and all other staffs of GENETUP, for their help and co-operation during my research work.

I want to express my thankful to all of the patients, who were included in my research, without whose co-operation I never complete my research work. I am eternally grateful and wish them to get well soon.

I would also like to express my heartfelt gratitude to director and all staffs of Everest International Clinic and Research Center for providing associated reagents, equipments and laboratory assistance.

I would also like to express my sincere gratitude to Prof. Dr. Y. Suzuki, Dr. T. Yoda and all Japanese team for their supplies of LAMP reagents as well as technical assistance.

I extend my thanks to Dr. Sher Bahadur Pun, Mr. Ajay Poudel, Mr. Balram Adhikari, and Mr. Kiran Pandey for their help during study period.

Finally, I would like to express my deepest gratitude to my respected parents and family members for their blessing, understanding, continuous inspiration and encouragement to complete this work.

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ABSTRACT

Mycobacterium tuberculosis still remains a substantial global threat. The conventional biochemical tests for identification of mycobacterial species are time-consuming. Loop-Mediated Isothermal Amplification (LAMP) provides new possibilities of other tests requirements for direct detection of *M. tuberculosis, M. avium* complex and *M. kansasii* in sputum samples. This study was carried out from February 2009 to March 2010 based at German Nepal Tuberculosis Project, Everest International Clinic and Research Center in collaboration with Central Department of Microbiology and Osaka Perfectural Institute of Public Health Japan. A total of 135 (100%) sputum specimens (103 from new suspected pulmonary tuberculosis patients and 32 follow up MDR patients) were included in this study for comparative study of Microscopy, culture and LAMP. Among them, 60 (44.4%) were microscopy positive. Similarly 68 (50.3%) sputum specimens were positive by culture and 71 (52.5%) sputum specimens were positive by LAMP. Out of 71 (100%) total LAMP positive cases, 70 (98.5%) were positive with *M. tuberculosis* primer and remaining 1(1.4%) was positive with M. intracellular primer. None of the *M. avium* and *M. kansasii* cases were found from the samples that were included in this study.

With reference to microscopy result, the sensitivity and specificity of LAMP were 96.6%and 82.6% respectively. Predictive value of positive test was 81.7%, predictive value of negative test was 96.8%, percentage of false negative was 3.3% and percentage of false positive was 17.3%.Similarly, while comparing the LAMP result with culture as gold standard, the sensitivity of LAMP was 97.5%, specificity was 92.5%, predictive value of positive test was 92.9%, predictive value of negative test was 96.8%, percentage of false positive was 7.4% and percentage of false negative was 2.9%.

This study showed that LAMP is sensitive and specific molecular technique, which can be used effectively for the diagnosis thus facilitating the effective treatment and case management of tuberculosis and other atypical mycobacterial infection.

Key words: *M. tuberculosis, M. avium* complex, *M. kansasii*, LAMP, TB, MAC-PD, MK-PD, Sputum

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LIST OF ABBREVIATIONS

A	:	Adenine
AFB	:	Acid fast bacilli
BCG	:	Calmette-Guerin Bacilli
bp	:	base pair
C	:	Cytosine
d ATP	:	2'-deoxyadenosine 5'-triphosphate
d TTP	:	2'-deoxythymine 5'-triphosphate
d CTP	:	2'-deoxycytidine 5'triphosphate
d GTP	:	2'-deoxyguanosine 5-'triphosphate
d NTPs	:	Deoxyribonucleoside triphosphates
DNA	:	Deoxyribonucleic acid
DOTS	:	Directly Observed Treatment Short Course Therapy
ELISA	:	Enzyme Linked Immunosorbent Assay
EPTB	:	Extra pulmonary Tuberculosis
FD	:	Fluorescence Dye
G	:	Guanine
HIV	:	Human Immuno-Deficiency Virus
IUATLD	:	International Union Against Tuberculosis and Lung Disease
KDa	:	Kilo Dalton
LAMP	:	Loop Mediated Isothermal Amplification
L-J	:	Lowenstein-Jensen Medium
MAC	:	*Mycobacterium avium complex*
MAC-PD	:	*Mycobacterium avium complex* –Pulmonary Disease
MAV	:	*Mycobacterium avium*
MK	:	*Mycobacterium kansasii*
MK-PD	:	*Mycobacterium kansasii*-Pulmonary Disease
MTB	:	*Mycobacterium tuberculosis bacilli*
MOTT	:	Mycobacteria Other Than Tuberculosis
NAA	:	Nucleic Acid Amplification
NTC	:	National Tuberculosis Center

NTP	:	National Tuberculosis Programme
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
PPD	:	Purified Protein Derivative
PTB	:	Pulmonary Tuberculosis
rpm	:	Revolution per Minute
TB	:	Tuberculosis
TST	:	Tuberculin Skin Test
WHO	:	World Health Organization
Z-N	:	Ziehl Neelsen