USE OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DIRECT DETECTION OF *Mycobacterium* IN SPUTUM

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(MEDICAL)

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ABSTRACT

Most first line anti-tuberculosis drugs have less invitro activity against *M. avium, M. intracellular* and *M. kansasii*. Therefore rapid species identification and proper use of drugs are key requirements for the effective treatment and case management of tuberculosis as well as *Mycobacterium avium* complex-pulmonary disease (MAC-PD) and *Mycobacterium kansasii*-pulmonary disease (MK-PD). The development and evaluation of new diagnostic technique, which can diagnose causative agent in simple and rapid way, is the necessity of this century. Loop-Mediated Isothermal Amplification (LAMP) provides new possibilities of above requirements for direct detection of *M. tuberculosis, M. avium* complex and *M. kansasii* in sputum samples.

This study was carried out from October 2005 to September 2006. A total of 190 (129 from 43 new suspected pulmonary tuberculosis patients and 61 from 61 follow up patients) sputum samples were included in this study. All these samples were further processed for flurochrome staining but only 130(69 from new suspected pulmonary tuberculosis patients and 61 from follow up patients) sputum specimens were subjected to culture and LAMP. Thus 130 sputum specimens were included in this study to compare them with microscopy, culture and LAMP. Among them 50(38.46%) were found to be positive by flurochrome staining, culture and LAMP. Similarly 48(36.92%) samples were negative by all diagnostic methods. 1(0.77%) microscopy and culture positive sample was negative by LAMP. Similarly 3(2.31%) microscopy and LAMP positive cases were negative by culture. 3(2.31%) culture positive cases were negative by both Microscopy and LAMP. 8(6.16%) culture negative cases were positive by LAMP where as 17(13.07%) Microscopy negative samples were positive by culture and LAMP.

Out of 78(100%) total LAMP positive cases, 76(97.44%) were positive with *M. tuberculosis* primer and remaining 2(2.56%) were 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.

While comparing the LAMP results with gold standard culture, the sensitivity, specificity, predictive value of positive test, predictive value of negative test, percentage of false negative and percentage of false positive of LAMP were found to be 94.36%, 81.36%, 85.90%, 92.31%, 5.63% and 18.64% respectively. Similarly, LAMP had sensitivity 98.14% and specificity 67.11% while compare with microscopy.

Therefore, LAMP is sensitive and specific molecular technique, which can be used effectively for the diagnosis of clinically, microscopically, and culturally confusing cases thus facilitating the effective treatment and case management of tuberculosis and other atypical mycobacterial infection. Due to its easy operation and rapid amplification efficiency, it can be used in well-equipped laboratories for clinical use if sample preparation, nucleic acid extraction and cross-contamination controls are addressed.

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

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LIST OF ABREVIATIONS

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	:	Deoxyrionucleoside triphosphates
DNA	:	Deoxyribonucleic acid
FD	:	Fluorescence Dye
G	:	Guanine
C	:	Cytosine
HIV	:	Human Immuno-Deficiency Virus
IFN	:	Interferon
KDa	:	Kilo Dalton
LAMP	:	Loop-Mediated Isothermal Amplification
L-J	:	Lowenstein-jensen Medium
LTBI	:	Latent Tuberculosis Infection
MAC	:	*Mycobacterium avium* complex
MAC-PD	:	*Mycobacterium avium* complex-Pulmonary Disease
MAV	:	*Mycobacterium avium*
MIN	:	*Mycobacterium intracellulare*
MK	:	*Mycobacterium kansasii*
MK-PD	:	*Mycobacterium kansasii*- Pulmonary Disease
MTB	:	*Mycobacterium tuberculosis* bacilli
MOTT	:	Mycobacteria Other Than Tuberculosis

NAA	:	Nucleic Acid Amplification
NALC	:	N-acetyl-L-cysteine
NTC	:	National Tuberculosis Center
NTP	:	National Tuberculosis Programme
NTM	:	Non tuberculosis Mycobacteria
NK	:	Natural killer cell
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
PTB	:	Pulmonary Tuberculosis
RFLP	:	Restriction fragment length polymorphism
rpm	:	Revolution Per Minute
TB	:	Tuberculosis
WHO	:	World Health Organization
Z-N	:	Ziehl-Neelsen