COMPARATIVE STUDY OF POLYMERASE CHAIN REACTION (PCR) AND LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR DIRECT DETECTION OF *Mycobacterium tuberculosis* IN SPUTUM

A

DISSERTATION

PRESENTED 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

BY

BINITA KOIRALA

DEPARTMENT OF MICROBIOLOGY

TRIBHUVAN UNIVERSITY

KIRTIPUR, KATHMANDU, NEPAL

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BINITA KOIRALA

2008

RECOMMENDATION

This is to certify that **Ms. Binita Koirala** has completed this dissertation work entitled " **Comparative study of Polymerase Chain Reaction (PCR) and Loop-Mediated Isothermal Amplification (LAMP) for Direct Detection of *Mycobacterium tuberculosis* in Sputum**" as a partial fulfillment of M. Sc. degree in Microbiology under our supervision. To our knowledge this work has not been submitted for any other degree.

Dr. Anjana Singh Associate Professor Central Department of Microbiology Tribhuvan University Kirtipur, Kathmandu, Nepal	Dr. Basu Dev Pandey Senior Medical Officer Sukra Raj Tropical and Infectious Disease Hospital Teku, Kathmandu, Nepal	Dr. Bhawana Shrestha Project chief, German-Nepal Tuberculosis Project (GENETUP), Kalimati, Kathmandu, Nepal	Mr. Bishwa Raj Sapkota, Scientist, Mycobacterial Research Laboratory Anandaban Hospital, Lele, Lalitpur, Nepal

Date…….

CERTIFICATE OF APPROVAL

On the recommendation of **Dr. Anjana Singh, Dr. Basu Dev Pandey, Dr. Bhawana Shrestha** and **Mr. Bishwa Raj Sapkota,** this dissertation of **Ms. Binita Koirala** is approved for the examination and is submitted to Tribhuvan University in Partial fulfillment of the requirements for M. Sc. degree in Microbiology.

Date: ……..

_____

Dr. Anjana Singh
Head
Central Department of Microbiology
Tribhuvan University
Kirtipur, Kathmandu
Nepal

BOARD OF EXAMINERS

Recommended by:

_____

Dr. Anjana Singh
Supervisor

_____

Dr. Basu Dev Pandey
Supervisor

_____

Dr. Bhawana Shrestha
Supervisor

_____

Mr. Bishwa Raj Sapkota
Supervisor

Approved by:

_____

Dr. Anjana Singh
Head of Department

Examined by:

_____

Dr. Keshab Bhakta Shrestha
Former Director, NTC, and
Medical Director
Martyr Memorial Hospital
External Examiner

_____

Mr. Binod Lekhak
Internal Examiner

Date: ……

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……..

Date: …… Binita Koirala

ABSTRACT

The risk of spread of infection and emmergence of drug-resistant strain has created the need for a rapid, sensitive and specific diagnostic test for tuberculosis. In addition, clinically suspicious cases that do not give positive result in conventional laboratory test need the development and evaluation of new diagnostic technique, which can identify the etiological agent in rapid way.

The study was carried out from October 2006 to November 2007 based at German Nepal Tuberculosis Project, Mycobacterial Research Laboratory, Anandaban Hospital and Everest International Clinic and Research Center in collaboration with Central Department of Microbiology (CDMTU) and Osaka Perfectural Institute of Public Health, Japan. A total of 106 (53 fluorochrome staining positive and 53 fluorochrome staining negative) sputum samples were collected in this study. Out of 53 fluorochrome staining positive samples, all the samples 53 (100%) were positive on culture on Ogawa medium and 51 (96.22%) were found to be positive in both PCR and LAMP. Two (3.77%) PCR and LAMP negative samples were positive in culture.

Similarly, of 53 fluorochrome staining negative samples, 4 (7.54%) samples were positive and 45 (84.90%) were negative in all culture, PCR and LAMP. There was 1 (1.88%) sample which was positive by LAMP and PCR but culture negative and 3 (5.66%) samples were positive only by PCR but negative by culture and LAMP.

While comparing the microscopy results with culture as gold standard, the sensitivity, specificity, positive and negative predictive values were 92.98%, 100%, 100% and 92.45% respectively, however, these values for PCR were 96.49%, 91.83%, 93.22% and 95.74% respectively with reference to culture. Similarly, the sensitivity, specificity, positive and negative predictive values for LAMP with reference to culture were 96.49%, 97.95%, 98.21% and 96% respectively, however, with reference to PCR, these values were 94.91%, 100%, 100% and 94% respectively.

This study showed that PCR and LAMP could be a possible diagnostic tool for the confirmation of the smear negative cases that show clinical symptoms of TB. Due to its easy operation without sophisticated equipment, LAMP will be simple enough to use in small scale hospitals, primary care facilities, and clinical laboratories in developing countries if the remaining issues such as sample preparation, nucleic acid extraction and cross-contamination controls are addressed.

Key words: Sputum, *M. tuberculosis*, DNA, LAMP, PCR, Sensitivity, Specificity

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LIST OF ABBREVIATIONS

A	:	Adenine
AFB	:	Acid Fast Bacilli
BCG	:	*Bacilli Calmette-Guerin*
bp	:	base pair
C	:	Cytosine
d ATP	:	2'-deoxyadenosine 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
LTBI	:	Latent Tuberculosis Infection
MHC	:	Major Histocompatibility Complex
MOTT	:	Mycobacteria other Than Tuberculosis
NAA	:	Nucleic Acid Amplification
NaLC	:	N-acetyl-L-cysteine
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
SAARC	:	South Asian Association of Regional Corporation
STC	:	SAARC Tuberculosis Center
T	:	Thymine
TB	:	Tuberculosis
TST	:	Tuberculin Skin Test
WHO	:	World Health Organization
Z-N	:	Ziehl Neelsen

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