

**SCREENING OF *MYCOBACTERIUM TUBERCULOSIS* BY  
SELECTIVE INHIBITION WITH PARA-NITROBENZOIC ACID,  
ITS CYTOCHEMICAL STAINING AND DRUG SUSCEPTIBILITY  
TO PRIMARY ANTI-TUBERCULAR DRUGS**

A

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Master of Science in Microbiology

(Medical)

by

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## RECOMMENDATION

This is to certify that Mr. Sujay Nepali has worked under our supervision and guidance on the thesis entitled “**Screening of *Mycobacterium tuberculosis* by selective inhibition with para-nitrobenzoic acid, its cytochemical staining and drug susceptibility to primary anti-tubercular drugs**” as a partial fulfillment of M.Sc. Degree in Microbiology. To the best of our knowledge this is an original research work of him and has not been submitted for any other degree.

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On the recommendation of Associate Professor **Dr. Prakash Ghimire, Ph.D.** and **Mr. Dhruva Kumar Khadka** this dissertation work by **Mr. Sujay Nepali**, entitled **“Screening of *Mycobacterium tuberculosis* by selective inhibition with para-nitrobenzoic acid, its cytochemical staining and drug susceptibility to primary anti-tubercular drugs”** has been approved for the examination and is submitted to Tribhuvan University in partial fulfillment of the requirement for M.Sc. Degree in Microbiology.

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## ABSTRACT

This diagnostic study was conducted at National Tuberculosis Centre (NTC), Thimi, Bhaktapur from September 2006 to June 2007; with the objectives of screening of *Mycobacterium tuberculosis* using para-nitrobenzoic acid (PNB) added to the culture medium, the virulence testing using neutral red and response of the isolates to primary set of anti-tubercular drugs.

Of 857 sputum samples taken, 68.3% were from male and 31.7% from female. Out of total, 28.7% were positive for acid fast bacilli (AFB) by fluorescence microscopy, of which 78.45% were male and 21.55% were female. Out of total smear positive cases, 87% were also positive in culture on Ogawa medium.

All the Ogawa culture positive samples failed to grow on LJ medium containing PNB. However all the isolates were neutral red test positive. The drop catalase test and 68°C labile catalase test were also used for the confirmation of the isolated *M. tuberculosis*.

The proportion method used for the drug susceptibility test showed that 43.46% of the culture positive isolates were sensitive to all four primary anti-tubercular drugs. The resistance rate to one drug was found in 11.21%, to two drugs in 14.95%, to three drugs in 13.55% and to four drugs in 16.82% of the isolates. Multi-drug resistance (MDR) was observed in 28.97% of the isolates.

Hence, the use of PNB in culture media is useful in the screening of *M. tuberculosis* along with its differentiation from non-tuberculous mycobacteria. Similarly, neutral red, a biochemical test used for identification of other bacteria, can be used for the virulence testing of *M. tuberculosis* – the method which is easy and rapid. The susceptibility testing result can be used as the guidance for proper treatment and in the management of MDR cases.

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## LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immuno Deficiency Syndrome
APC	Antigen Presenting Cell
AST	Antimicrobial Susceptibility Testing
BACTEC	Becton Dickinson and Company
BCG	Bacille Calmette Guéruin
CDC	Centre for Disease Control
CMI	Cell Mediated Immunity
CR	Conventional Receptor
CRP	C-Reactive Protein
DNA	Deoxyribo Nucleic Acid
DOTS	Directly Observed Treatment Short course
DST	Drug Susceptibility Test
EMB/E	Ethambutol
ESR	Erythrocyte Sedimentation Rate
E-test	Epsilometer-test
GLC	Gas Liquid Chromatography
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPA	Hybridization Protection Assay
HPLC	High Performance Liquid Chromatography
HSP	Heat Shock Proteins
ICT	Immuno Chromatographic Technique
IFN	Interferon
IL	Inter-Leukin
ImCRAC	Immuno-Cross-Reactive Antigen Compound
INH/H	Isoniazid

IS	Insertion Sequence
IUATLD	International Union Against Tuberculosis and Lung Diseases
LAMs	Lipoarabinomannans
LCR	Ligase Chain Reaction
LIPA	Line Probe Assay
LJ media	Lowenstein Jensen media
LTBI	Latent TB Infection
MDR	Multi Drug Resistant
MDR-TB	Multi Drug Resistant-Tuberculosis
MGIT	Mycobacterium Growth Indicator Tube
MHC	Major Histocompatibility Compound
MIC	Minimum Inhibitory Concentration
MOTT	Mycobacteria Other than Tuberculous bacilli
MTC	Mycobacterium Tuberculosis Complex
NALC-NaOH	N-Acetyl Cysteine-Sodium Hydroxide
NRAMP	Natural Resistance-Associated Macrophage Protein
NTC	National Tuberculosis Centre
NTM	Non-Tuberculous Mycobacteria
NTP	National Tuberculosis Programme
PCR	Polymerase Chain Reaction
PGRS	Polymorphic GC rich Repetitive Sequence
PNB	Para-NitroBenzoic acid
PPD	Purified Protein Derivative
PTB	Pulmonary Tuberculosis
PZA/Z	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RFP/R	Rifampicin
RNI	Reactive Nitrogen Intermediate
ROI	Reactive Oxygen Intermediate
SAARC	South Asian Association for Regional Cooperation

SEAR	South East Asia Region
SLDs	Second Line Drugs
SM/S	Streptomycin
SPSS	Statistical Package for Social Science
STC	SAARC Tuberculosis Centre
TB	Tuberculosis
TCH	Thiophen-2-Carboxylic acid Hydrazide
TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
WHO	World Health Organization
XDR	Extended Drug Resistant
ZN staining	Ziehl-Neelsen staining
Z-TSP	Zephiran-Tri-Sodium Phosphate