

**EVALUATION OF NITRATE REDUCTASE ASSAY FOR RAPID  
DETECTION OF DRUG RESISTANT TUBERCULOSIS**

A

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Tribhuvan University

In Partial Fulfillment of the Requirements for the Award of the Degree of  
Master of Science in Microbiology  
(Medical)

by

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## RECOMMENDATION

This is to certify that Mr. Ranjit kumar Sah has worked under our supervision and guidance on the thesis entitled “EVALUATION OF NITRATE REDUCTASE ASSAY FOR RAPID DETECTION OF DRUG RESISTANT TUBERCULOSIS” as a partial fulfillment of M.Sc. Degree in Microbiology. To the best of our knowledge this work has not been submitted for any other degree.

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## ABSTRACT

Due to the emergence of multidrug-resistant tuberculosis (MDR-TB), there is an urgent demand for simple, rapid and inexpensive methods of detecting drug resistant tuberculosis for effective treatment, particularly in low-income countries. This study has the objective to evaluate nitrate reductase assay (NRA) efficacy for streptomycin, isoniazid, rifampicin and ethambutol susceptibility testing of *Mycobacterium tuberculosis* strains. This prospective study was carried out at National TB Reference Laboratory and SAARC TB and HIV/AIDS Centre, Thimi, Bhaktapur, Nepal from November 2009 to May 2010. A total of 113 clinical isolates of *M. tuberculosis* were tested for four first line antitubercular drugs by nitrate reductase assay and were compared with standard proportion method. The sensitivity and specificity of NRA were 98.1% and 100% for isoniazid, 95.1% and 98.6% for rifampicin, 91.4% and 94.9% for streptomycin, and 78.6% and 97.9% for ethambutol respectively. Agreement between NRA and proportion method were 99.1%, 97.3%, 93.8%, 95.6% for isoniazid, rifampicin, streptomycin and ethambutol respectively. Results were available in 7-14 days by NRA as compared to proportion method which takes 4-6 wk. NRA is reliable for susceptibility testing of isoniazid and rifampicin, the two most important drugs for the treatment of tuberculosis and is also easy to perform and inexpensive. In addition, the reduction in the time necessary to obtain susceptibility results is of fundamental importance.

**Key words:** Drug susceptibility, *Mycobacterium tuberculosis*, nitrate reductase assay, Proportion method

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## LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
AIDS	Acquired Immuno Deficiency Syndrome
BACTEC	Becton Dickinson and Company
BCG	Bacille Calmette Guéruin
BTS	British Thoracic Society
CDC	Centre for Disease Control
CPC	Cetyl Pyridinium Chloride
DNA	Deoxyribo Nucleic Acid
DOTS	Directly Observed Treatment Short course
DOT-SCC	Directly Observed Therapy with Short-Course Chemotherapy
DRS	Drug Resistance Surveillance
DST	Drug Susceptibility Testing
EMB/E	Ethambutol
EPTB	Extra Pulmonary Tuberculosis
E-test	Epsilometer-test
ETH	Etionamide
FDC	Fixed Dose Combination
GI	Growth Index
HIV	Human Immunodeficiency Virus
ICL	IsoCitrate Lyase
INH/H	Isoniazid
IS	Insertion Sequence
IUATLD	International Union Against Tuberculosis and Lung Diseases
LAMs	Lipoarabinomannans
LiPA-Rif	Line Probe Assay- Rifampicin
LJ	Lowenstein Jensen
MABA	Microplate Alamar Blue Assay

MDR	Multi Drug Resistant
MDR-TB	Multi Drug Resistant-Tuberculosis
MGIT	Mycobacterium Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MOTT	Mycobacteria Other than Tuberculous bacilli
MTC	Mycobacterium Tuberculosis Complex
MTT	3-(4,5 diMethylThiazol-2-yl)-2,5-diphenyl Tetrazolium bromide
NAD	Nicotinamide Adenine Dinucleotide
NALC-NaOH	N-Acetyl Cysteine-Sodium Hydroxide
NPV	Negative Predictive Value
NR	Nitrate Reductase
NRA	Nitrate Reducataase Assay
NTC	National Tuberculosis Centre
NTM	Non-Tuberculous Mycobacteria
NTP	National Tuberculosis Programme
PCR	Polymerase Chain Reaction
PM	Proportion method
PNB	Para-NitroBenzoic acid
POA	Pyrazinoic Acid
PPV	Positive Predictive Value
PTB	Pulmonary Tuberculosis
PZA/Z	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RMP/R	Rifampicin
RNA	Ribo Nucleic Acid
RR	Resistance Ratio method
SAARC	South Asian Association for Regional Cooperation
SEAR	South East Asia Region
SM/S	Streptomycin
STAC	SAARC Tuberculosis and HIV/AIDS Centre

TB	Tuberculosis
TCH	Thiophene 2 Carboxylic acid Hydrazide
WHO	World Health Organization
XDR	Extended Drug Resistant
XDR-TB	Extended Drug Resistant Tuberculosis
ZN	Ziehl-Neelsen

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resistance)



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