

**Evaluation of rapid molecular drug susceptibility
testing for Mycobacterium tuberculosis**

Thesis

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LIST OF ABBREVIATIONS

AFB	Acid-fast bacillus
AAM	Alternatively activated macrophage
AM	Amikacin
ACP	Acyl carrier protein
AIDS	Acquired immunodeficiency syndrome
ATP	Adenosine triphosphate
ATS	American Thoracic Society
CAM	Classically activated macrophage
CLSI	Clinical and Laboratory Standards Institute
CM	Capreomycin
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
DW	Distilled water
EMB	Ethambutol
ETH	Ethionamide
Fig	Figure
FLQS	Floroquinolones
G-CSF	Granulocyte colony stimulating factor
GenoType MTBDRplus	GenoType <i>Mycobacterium tuberculosis</i> drug resistance first line
GenoType MTBDRsl	GenoType <i>Mycobacterium tuberculosis</i> drug resistance second line
HIV	Human immunodeficiency virus
HPLC	high performance liquid chromatographic

LIST OF ABBREVIATIONS	
HRM	High-resolution melting
IFN-γ	gamma-interferon
IL	Interleukin
INH	Isoniazid
IS	Insertion sequence
INOS	Inducible nitric oxide synthetase
KAN	Kanamycin
KH₂PO₄	Potassium dihydrogen phosphate
LAM	Lipoarabinomannan
LJ	Lowenstein-Jensen
MAS-PCR	Multiplex allele specific PCR
MDR	Multi drug resistance
MDR TB	Multidrug resistance tuberculosis
MGIT	Mycobacteria growth indicator tube
MHC	Major histocompatibility complex
MIC	Minimum inhibitory concentration
MTB	Mycobacterium Tuberculosis
MTBC	Mycobacterium Tuberculosis complex
MTD	Mycobacterium Tuberculosis Direct Test
MODS	Microscopic observation broth-drug susceptibility
MUT	Mutation
NAD	Nicotinamide adenine dinucleotide
NALC	N-acetyl-L-cysteine
NAAT	Nucleic acid amplification technology
NaOH	Sodium hydroxide

LIST OF ABBREVIATIONS	
NAP	Beta nitro alpha acetylamine beta hydroxy propiophenone
NO	Nitric oxide
NPV	Negative predictive value
OFX	Ofloxacin
PNB	Para-nitro benzoic acid
PCR	Polymerase chain reaction
PPV	Positive predictive value
PZA	Pyrazinamide
QRDR	Quinolone Resistance- Determining Region
R	Resistant
RIF	Rifampicin
RNIs	Reactive nitrogen intermediates
ROIs	Reactive oxygen intermediates
ROS	Reactive oxygen species
RRDR	Rifampicin resistance determining region
ST	Streptomycin
SOP	Standard Operation Procedures
TB	Tuberculosis
TCH	Thiophene-2 carboxylic acid hydrazide
TDM	Trehalose 6,6'-dimycolate
TGF-β	Transforming growth factor-beta
Th0	T-helper type 0 cells
Th1	T-helper cell type 1
Th2	T-helper cell type 2
TL7H11	Thin layer 7H11 agar
TNF-α	Tumor necrosis factor alpha

LIST OF ABBREVIATIONS	
TLR	Toll-like receptor
VIO	Viomycin
VOCs	Volatile organic compounds
WHO	World Health Organization
WT	Wild type
XDR TB	Extensively drug resistance TB
ZN	Ziehl Neelsen

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Introduction

Tuberculosis (TB) remains one of the leading causes of morbidity and mortality globally, focused principally, but not exclusively, in the non-industrialized world **(Migliori, et al. 2008)**.

The World Health Organization (WHO) and Stop TB Partnership called urgently for expanded access to culture and drug susceptibility testing (DST) in response to the spreading out of multidrug resistance TB (MDR TB) and extensively drug resistance TB (XDR TB) which are declared by the WHO as serious emerging threats to public health. This poses significant challenges for TB laboratory capacity and the need for faster DST methods **(WHO, 2007)**.

MDR TB, means resistance to at least isoniazid (INH) and rifampin (Rif), and XDR, means MDR plus resistance to amikacin (AM), kanamycin (KAN) or capreomycin (CM) and a fluoroquinolone (FLQs), and it is the most problematic form of resistance because treatment options are limited and the second-line drugs used for therapy are more toxic, less effective, more expensive, and must be administered for a longer period of time than standard first-line drug therapy **(Migliori, et al. 2008)**.

Conventional culture methods using egg- or agar-based media are still the most commonly used approach in many countries. To test for drug resistance, the standard methods using Lowenstein–Jensen (LJ) medium include the proportion method, the absolute concentration method and the resistant ratio method, which are well standardized with clinical isolates, at least for the major antituberculosis drugs **(Canetti, et al. 1969)**.

Conventional culture and DST on solid media is a slow process. In high income, low-incidence countries these systems have been supplemented (or replaced) by more rapid automated liquid culture systems such as BACTECT 960 system **(Somoskovi, et al. 2003)**.

Molecular assay techniques based on information about the genes involved in drug resistance represent a great step forward, as they provide results even more rapidly. Rapid molecular methods, including commercial or in-house DNA hybridization or amplification methods **(Somoskovi, et al. 2003)** which allow detection of TB and rifampin resistance (and for some assays, isoniazid resistance as well) in clinical samples within 1–2 days **(Bardarov, et al. 2003 and Hillemann, et al. 2007)**.

Conventional DST for XDR strains is performed sequentially in a two-step procedure beginning with a culture and first-line drug testing, proceeding to further drug testing in the case of multidrug resistance. The time needed for testing, even with the most rapid

liquid methods, is still around 1 week per test, constrained by the relatively slow growth of *M. tuberculosis* (**Antonova, et al. 2008**). The required time can be shortened by fast molecular methods to 1 day per test (**Bang, et al. 2006** and **Hillemann, et al. 2006**). Resistance to FLQs, AM-CM, and ethambutol (EMB) in *M. tuberculosis* is most frequently attributed to mutations in the *gyrA*, *rrs*, and *embB* genes, respectively. First investigations have shown that by targeting mutations in codons 90, 91, and 94 in the *gyrA* gene, approximately 70 to 90% of all FLQ-resistant strains can be correctly detected (**Maus, et al. 2005** and **Ling, et al. 2008**). Previous reports have linked mutations A1401G, C1402T, and G1484T in the *rrs* gene to AM, CM, and KAN resistance (**Alangaden , et al. 1998** and **Maus, et al. 2005**), each of them being responsible for a specific resistance pattern. Mutations G1484T and A1401G were found to cause high-level resistance to all drugs, whereas C1402T causes resistance to only CM and KAN. Furthermore, mutations at *embB* codon 306 are found in 30 to 68% of EMB-resistant clinical strains (**Plinke, et al. 2006** and **Safi, et al. 2008**).

PCR-based techniques provide new possibilities for the rapid diagnosis of first- and second-line drug resistance; however, not all mycobacterial laboratories have access to DNA-sequencing facilities. As an alternative, DNA strip assays for the detection of