The Development of a Rapid Diagnostic System for Difficult to Culture Human Pathogens

By

Balkis A Talip, B.Sc.(Hons), M.Sc.

In partial fulfilment of the requirements of the degree of Doctor of Philosophy

May 2013

School of Biomedical Sciences

Faculty of Life and Health Sciences

University of Ulster

(I confirm that the word count of this thesis is less than 100,000)

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

ABSTRACT

The World Health Organization (WHO) has outlined a TB-free World strategy that aims to reduce tuberculosis prevalence by 2015. That could be achieved through the development of a specific and rapid detection system. In countries with a high incidence of tuberculosis, detection is solely based on sputum smear microscopy using Ziehl-Neelsen stain and chest radiography. This is followed by cultivation, which requires up to 12 weeks for confirmation. The diagnostic test available is insensitive, laborious, lacks specificity and does not even give unequivocal proof of infection. Consequently, this study was conducted to obtain confirmatory diagnostic information from Ziehl-Neelsen stained smears on glass slides. The fast-grower, non-pathogenic *Mycobacterium smegmatis* was used as the model organism due to similarity to the pathogenic strain, *Mycobacterium tuberculosis*. Laser capture microscopy (LCM) is primarily used as a microdissection tool in studies involving tissues and membranes. This technology was able to isolate intact individual cells fixed on archived glass slides regardless of how the smears and staining have been performed, confirmed by transmission electron microscopy (TEM) observation. Typically between 100 – 1,000 catapulted cells are enumerated using flow cytometry (FCM). A series of DNA extraction techniques which are heat-shock, heat-shock followed by ethanol precipitation and QIAamp® DNA Micro kit were compared and optimized for the lowest number of post-catapult cells. To maintain the detection of lowest number of catapulted cells, the *rpoB*-gene specific primers were designed for amplification using nested real-time PCR. Routinely, this system was able to detect as few as 30 catapulted cells per assay. This result demonstrated that it is possible to isolate the bacteria from glass slides and subsequently perform downstream molecular applications regardless of any

inhibitory factors. In conclusion, we strongly recommend that this system may offer improved specificity and speed of tuberculosis detection with lower risk of exposure to infection through the use of stained slides.

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
CDC	Centres for Diseases Control and Prevention
cDNA	copy DNA/complementary DNA
C_q	Quantification cycle
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside Triphosphate
FCM	Flow cytometry
G	gram(s)
HIV	Human immunodeficiency virus
L	litre(s)
LCM	Laser Capture Microscope/Laser Capture Microscopy
MDA	Multiple displacement amplification
mg	milligram(s)
ml	millilitre(s)
mm	millimetre(s)
mM	millimolar
mRNA	messenger RNA
µg	microgram(s)
µl	microlitre(s)
µm	micrometer(s)
NAA	Nucleic Acid Amplification Assay
NCBI	National Center for Biotechnology Information
ng	nanogram(n)
PBS	phosphate buffered saline
PCR	polymerase chain reaction

PI	Propidium Iodide
PCM	Post-catapult material(s)
PMIT	Photomultiplier tubes
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
rpoB	gene that encodes β-subunit of bacterial RNA polymerase
RT-PCR	Reverse Transcription PCR
ddH_2O	double distilled water
sp./spp.	species
Taq	*Thermus aquaticus*
TBE	tris-borate-EDTA
TE	Tris-EDTA
TO	Thiazole orange
tRNA	total RNA
U	unit
WHO	World Health Organization

TABLE OF CONTENTS

Chapter 1

General introduction.

1.1 An overview of the global tuberculosis (TB) incidence.

Tuberculosis is a major health problem caused by *Mycobacterium tuberculosis* complex (MTBC) with many new cases reported each year which claim almost 2 million deaths (Dagnew et al. 2012, Dias et al. 2012). Many countries are affected by tuberculosis (Table 1.1). In 2011, 85% of cases were reported from Africa and Asia with the major prevalence, 59%, contributed from India and China alone. Meanwhile, a small proportion of tuberculosis incidence reported was by the Americas (3%), the European region (4.3%), Eastern Mediterranean (7.7%) and Western Pacific (21% (reported in 2010) (Dias et al. 2012, Small 2012). Tuberculosis is ranked as the eighth leading cause of death in low- and middle income countries. However, it becomes the third biggest killer after HIV/AIDS and ischemic heart disease among adult populations (age 15 to 59) (Lessells et al. 2011, Noens et al. 2011, Balakrishnan et al. 2012). *M. tuberculosis* infection is transmitted by the inhalation of infected aerosol droplets generated by people with pulmonary disease, through coughing. Transmission by other methods has not been reported.

Although tuberculosis is curable, the available treatment regimes slow the infection rate but do not eliminate this infection from the population. This is indicated when reported tuberculosis cases continued to increase every year even though the number per 100,000 population decreased by 1% (Dias et al. 2012, Fitzpatrick et al. 2012). Treatment, using first-line drugs to treat *M. tuberculosis* among susceptible patients, does have an effect within 6 months. For the non-susceptible patient, second-line drug treatments are introduced which are used to treat multidrug-resistant tuberculosis (MDR-TB) strains (Ahmad 2011). Despite the presence of second-line drugs which are able to overcome non-susceptible patients,

the high cost and side effects of their use result in cases of MDR-TB not being eradicated.

Tuberculosis cases also relate to the spread of HIV/AIDS. In the 22 high-burden countries (HBCs) classified by WHO (accounting for 80% of tuberculosis cases) showed the tuberculosis cases reported from 1990 to 2011 are mostly contributed from the HIV-positive patient (Figure 1.1) (Dias et al. 2012, Fitzpatrick et al. 2012). According to WHO, one in four deaths from tuberculosis is HIV related. The diagnoses of HIV-positive patients are more difficult to treat as opposed to non-HIV-positive patients. In 2007, almost 1.37 million new tuberculosis cases were reported among HIV infected people alone. In addition, due to the low curable rate of people living with HIV, the mortality rate increased among these cases. The highest number of HIV-related tuberculosis cases peaked in Africa during the 1980s and steadily increased to 2004 (Blanc et al. 2009). The extensive treatment and monitoring then slowed the epidemic. Although new tuberculosis cases had reportedly decreased among the WHO region, the absolute numbers of cases increased along with the growth of population.

From 2009 to 2011, the majority of tuberculosis incidence and deaths were reported from less-developed regions of world countries with the highest death rates recovered from the poorest region of Africa, India, China and Southeast Asia (approximately 20 to 40 per 100,000 population) (Small 2012, Fitzgerald, Sterling & Haas 2010). This may be due to low income countries having limited access to health-care systems, high exposure to unhealthy dwelling, poor-nutrition, HIV infection, diabetes mellitus, and unhealthy life styles like smoking and drug abuse (El Khechine et al. 2009, Balakrishnan et al. 2012). Dating from 1990, the post-communist Eastern European region and former Soviet Union countries, were also

known as high tuberculosis burden areas where reported cases increased until 1998 (approximately 20 to 40 rates per 100,000 population). There was a decline of 8.5% in mortality rate shown from 1998 to 2011 (Dias et al. 2012). This trend has been related to improvements in political and socio-economic status of former Soviet Union dependents. Globally, mortality rates of tuberculosis are successfully decreasing as reported in 2011 among 22-HBCs (Figure 1.2).

The rise of MDR-TB and extensively drug-resistant TB (XDR-TB) cases have become a major concern of the WHO. This may be due to the determination of successful current strategic planning to control the spread of disease. There were an estimated 630,000 cases of MDR-TB among the world's 12 million tuberculosis cases reported in 2011. The actual number of cases and incidence trend is currently under investigation (Dias et al. 2012). Therefore the assessment of data for each country is still not fully accessible. However, an estimated number of MDR-TB cases reported, based on region, has been provided by WHO. Data was obtained from affected countries (Table 1.2). This surveillance data is crucial due to the chronic effects of MDR-TB infection.

Existing diagnostic methods for MDR-TB are slower and take longer to perform in comparison to diagnostics for MTBC common infection. Second-line drug treatment for MDR-TB is expensive due to the long treatment regime required to ensure effectiveness and prevent recurring infections. Although the death rate recorded in the WHO region from 1990 to 2011 has decreased, the actual number of deaths from HIV-related tuberculosis cases still may be greater than recorded. This may be due to no tuberculosis diagnosis from HIV patients but in such cases death is often not recorded as deaths due to tuberculosis. Many studies indicated that tackling the prominent factors of tuberculosis incidence allows the improvement of

tuberculosis controls. This involves many different intervention strategies which are largely dependent on political power and public awareness.

Table 1.1: An average estimation of the burden of disease caused by tuberculosis in 2011 reported by WHO. This summary has been adapted from the Global Tuberculosis Report 2012 (www.who.int/tb/publications/global_report/) and represents the rate per 100,000 population[a] (Dias et al. 2012).

Region	Population (thousand)	Mortality[b]	Prevalence	Incidence	HIV prevalence in incident TB cases (%)
Africa	857,382	26	293	262	41
America	943,039	2.2	35	28	17
Eastern Mediterranean	608,628	16	170	109	2.1
European	899,500	5.0	56	42	8.0
Southeast Asia	1,830,361	26	271	189	5.0
Western Pacific	1,808,797	6.9	138	92	3.1

Note:

[a] = The value is the point categorized as "best" estimation at lower and upper bounds of the 95% uncertainty interval.

[b] = Mortality excludes deaths among HIV-positive tuberculosis cases. Deaths among HIV-positive tuberculosis cases are classified as HIV deaths according to the International Classification of Diseases (ICD-10).

.

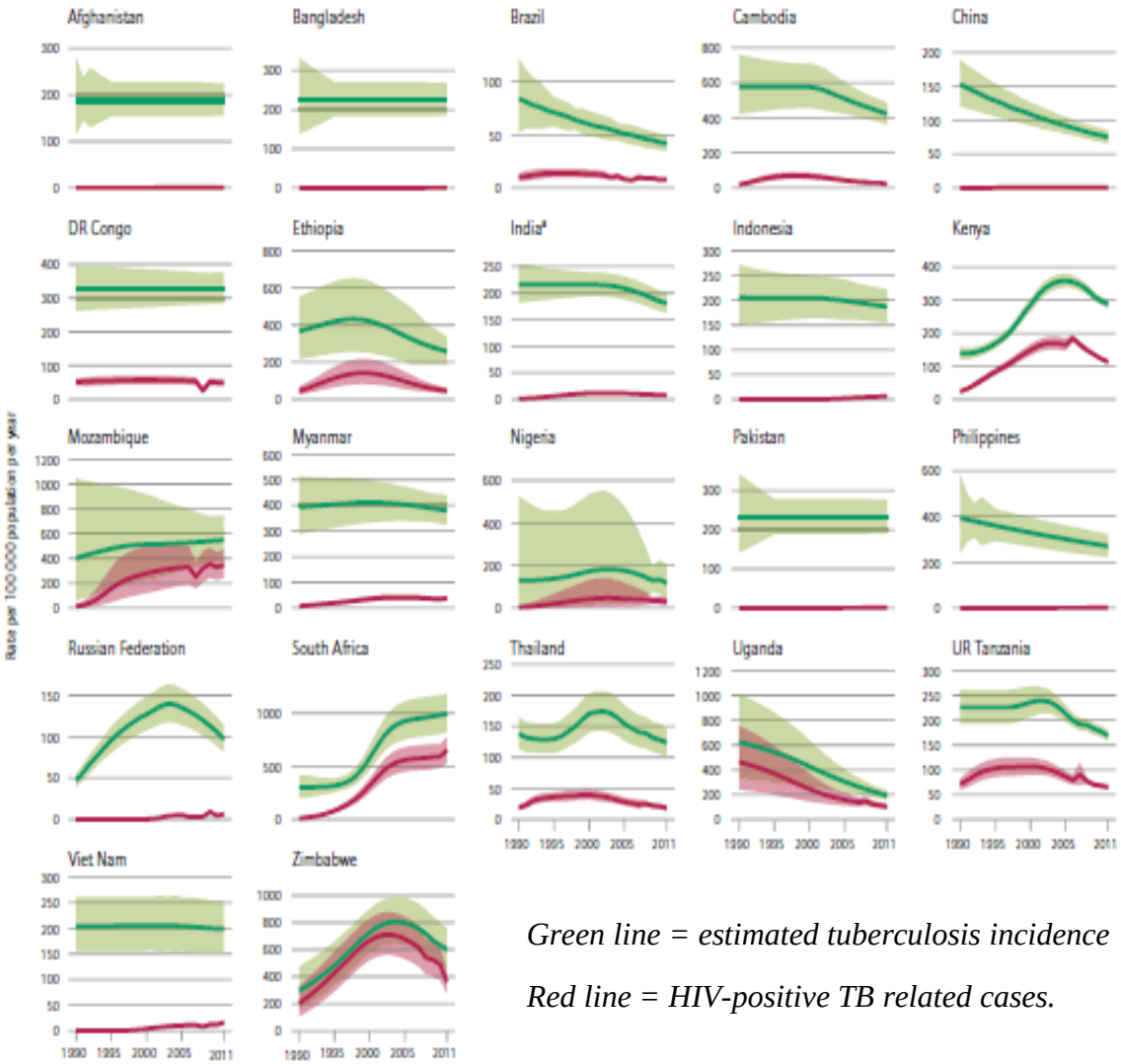


Figure 1.1: The incident rate of tuberculosis, reported from 1990 to 2011, from 22 classified high burden countries (HBCs) within the WHO region (Dias et al. 2012). These figures indicate that the tuberculosis incidence has been falling since the peak recorded in the mid-1990s among 22 HBCs. This was for most countries except for several regions of Africa as shown from the graph pattern.

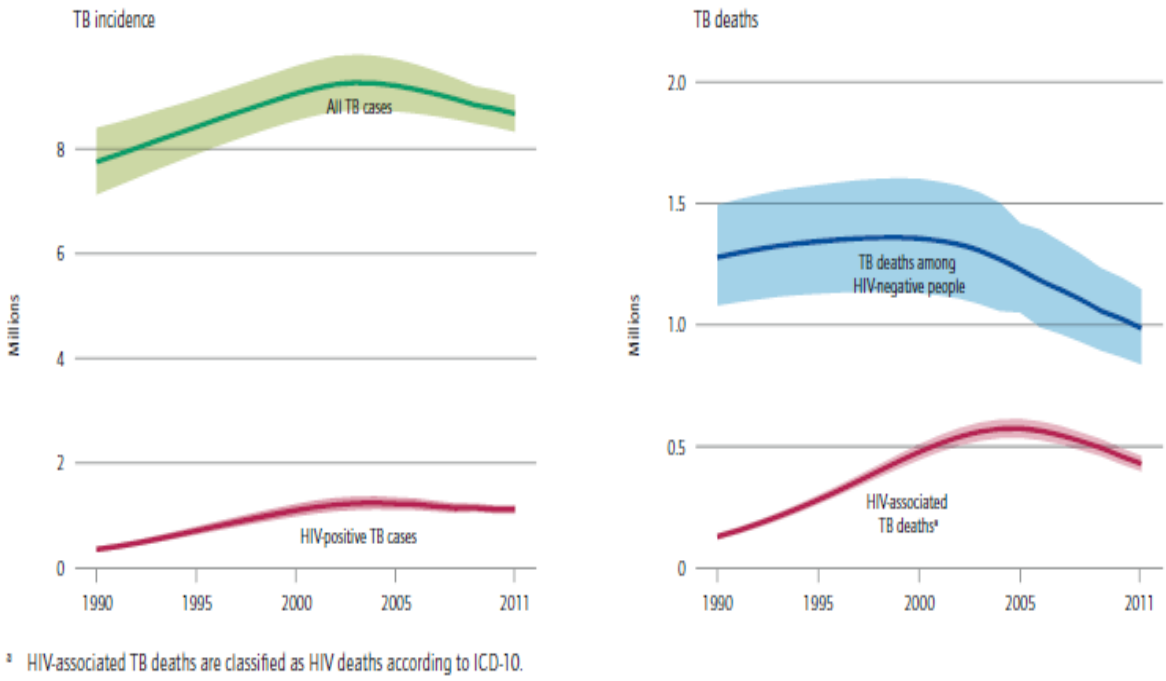


Figure 1.2: An estimated number of tuberculosis cases and death (in millions) reported between 1990 to 2011 globally (Dias et al. 2012). The falling rates of tuberculosis cases are shown globally from mid-2000. Despite the number of tuberculosis cases increasing, death rates have been reportedly declining every year.

Table 1.2: An estimation of MDR-TB cases reported in 2011 from WHO regions. This data was adapted from the Global Tuberculosis Report 2012 (www.who.int/tb/publications/global_report/). Direct measurements are still in progress therefore, the data supplied is an estimation of the actual incidence (Dias et al. 2012).

Region	Estimation of new TB cases with MDR-TB (%)	Confidence interval	Estimation of previous treated TB cases with MDR-TB (%)	Confidence interval
Africa	2.9	0.1-6.2	11	3.4-18
America	2.0	0.8-3.3	11	4.5-18
Eastern Mediterranean	3.4	0.1-10	30	6.9-53
European	5.1	10-20	44	40-49
Southeast Asia	2.1	1.8-2.5	16	12-19
Western Pacific	4.8	3.4-6.1	22	18-26

1.2 Description of the genus *Mycobacterium*.

Currently, there are almost 200 mycobacterial species that have been described by molecular techniques (Pfyffer, Palicova 2011). *Mycobacterium* is the only genus in the family of Mycobacteriacea. This aerobic bacterium contains various morphological shapes based on growth condition. Some species such as *Mycobacterium smegmatis* and *M. tuberculosis* are able to grow in a limited oxygen environment. It has typical rod-shape or slightly curve with cell dimensions of 0.2 to 0.6 µm by 1 to 10 µm (Pfyffer, Palicova 2011).

This genus cell wall is unique and contains a high proportion of a complex lipid that prevents access of common aniline dyes. This complex cell wall comprises an outer and inner compartment. The outer compartment contains protein and lipid. The lipid-linked polysaccharides (outer cell wall) consist of lipoarabinomannan (LAM), lipomannan, phthiocerol dimycocerosate, trehasole and phosphatidylinositol mannoside (Draper 1998, Hett, Rubin 2008). In addition, the sulfolipid is only present in *M. tuberculosis*. The outer compartment of the cell wall is soluble and has a role for interacting with the host immune system. The insoluble inner compartment of the cell wall consists of peptidoglycan (PG), arabinogalactan (AG) and mycolic acid (MA). These are linked together by covalent bonds and contribute to the core component of mycobacteria cell wall (Cole et al. 1998, Draper 1998, Toney et al. 2010).

Mycobacteria are grouped neither as Gram-positive nor Gram-negative bacteria. They have some unique qualities that are divergent from members of the Gram-positive group, which is that the cell wall contains mycolic acids. A prototypical Gram-positive bacterial cell wall contains a thick PG layer (10 to 20

sheets) with a size range of 20 to 80 nm for each sheet. This is contrary to a Gram-negative bacterial cell wall which consists of 1 to 10 thin layers (approximately 10 nm thickness of each layer) of PG (Figure 1.3). In contrast, a *Mycobacterium* cell wall consists of between 4 to 15 nm thickness of PG associated with teichoic acids, lipoteichoic acids and MAs which does not significantly show a distinctive layer of PG (Brennan 2003, Hett, Rubin 2008).

Mycobacteria are commonly described as acid-fast, implying that after staining they resist decolourization with acidified alcohol and also with strong mineral acid. The common staining procedure used is Ziehl-Neelsen staining (Pfyffer, Palicova 2011) which makes the bacteria stain red. The property of acid-fast, due to the waxy materials in the cell walls is particularly important to recognizing the *Mycobacterium* genus and primarily determines the permeability of cell walls. It is composed of α-alkyl and long β-hydroxyl-fatty acids (60 to 90 carbons for each chain) and is linked to AG. A study by Rao et al. (2005) has shown that a component of MAs (*trans*-cyclopropanation) suppresses inflammation caused by *M. tuberculosis* infection (Rao et al. 2005, Hett, Rubin 2008).

The *Mycobacterium* genus is also grouped as high G+C content bacteria with an average of 61 to 71 mol% G+C for all member species except 57 mol% for *M. leprae*. The genome size varies from species to species ranging from 4 to 7 million base pair (bp) (Table 1.3). Ninety percent (90%) of the genome represents coding regions that potentially encode for more than 6000 proteins (Cole et al. 1998, Zakham et al. 2011). Natural division and growth of *Mycobacterium* species differs based on slowly-growing to rapidly-growing characteristics. Slowly-growing *Mycobacterium* (SGM) requires more than 7 days for visible growth to appear as opposed to rapidly-growing *Mycobacterium* (RGM) which requires less than 7 days

when grown on Löwenstein-Jensen (L~J) medium. However, the RGM may require more than a week if grown from a clinical specimen (Brown-Elliott, Wallace 2011).

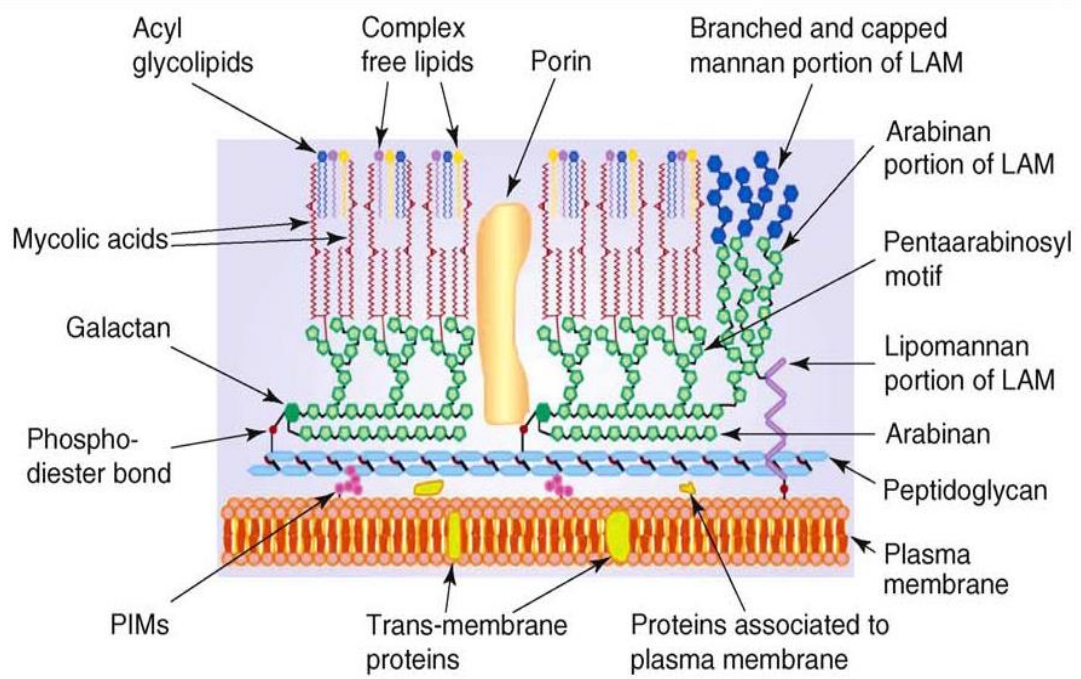


Figure 1.3: Schematic diagram of *Mycobacterium* showing the main components of the outer and inner layer of cell walls. The presence of mycolic acid in the complex of covalent-linked MA-AG-PG is a unique character for identification of the genus *Mycobacterium* and plays an important role as a permeability barrier (Medjahed, Gaillard & Reyrat 2010).

Table 1.3: List of selected *Mycobacterium* spp. showing variations in size and genome composition from species to species. This data was analysed and summarized from Genome Atlas Database (http://www.cbs.dtu.dk/services/GenomeAtlas).

Organism	Total size (bp)	Number of genes	Number of estimated proteins	G+C (%)	16S rRNA count	23S rRNA count	NCBI project ID
M. abscessus	5,090,491	4,941	1,030	64	1	1	15691
M. avium 104	5,475,491	5,120	1,069	69	1	1	88
M. bovis BCG str. Pasteur 1173P2	4,374,522	3,988	3,464	66	1	1	1173P2
M. leprae Br4932	3,268,071	3,720	1,202	58	1	1	31271
M. marinum M	6,660,144	5,452	1,222	66	1	1	16725
M. smegmatis str MC^2 155	6,988,209	6,716	5,614	68	2	2	92
M. tuberculosis H37Ra	4,419,977	4,034	1,096	66	1	1	18883
M. ulcerans Agy99	5,805,761	4,241	1,378	65	1	1	16230

1.3 Epidemiology and transmission.

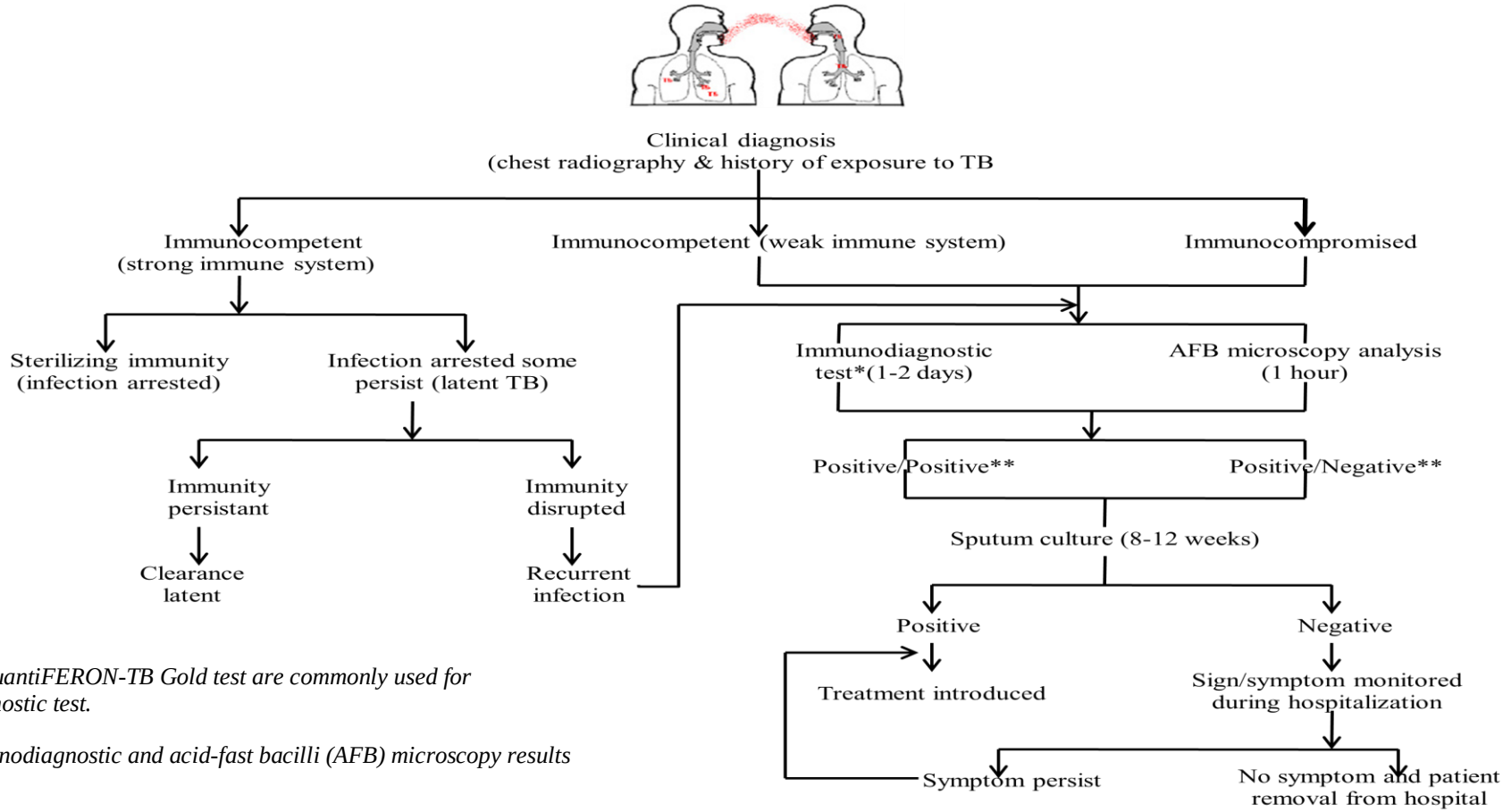
Members of the *Mycobacterium* genus are incapable of replicating in inanimate environments. For the MTBC and *M. leprae*, replication occurs in tissues of human and warm-blooded animals. The risk of infection is dependent on the load of the bacillus that has been inhaled, levels of infectiousness, person-to-person contacts and the immune level of the potential host. This air-borne pathogen is transmitted from an active pulmonary tuberculosis patient by expectoration (cough). The droplet nuclei, approximately 1 to 5 μm in size "meander" in the air and are transmitted to the susceptible person by inhalation (Figure 1.4).

The primary route of infection is the lungs. Tuberculosis can also infect other vital organs of human body such as kidney, spine and brain. Due to the relatively small size of the droplets inhaled into the lungs, the infection penetrates the defence system of bronchi and enters the terminal alveoli. These are then engulfed by alveolar macrophage and dendritic cells. The cell-mediated immune response arrests the multiplication of *M. tuberculosis* and stops infection. An infected person with a strong immune system is able to combat the infection within 2 to 8 weeks post-infection, when the active cell-mediated immune response alleviates multiplication of *M. tuberculosis*. However, in some patients, the tubercle bacilli are latently infected with asymptomatic appearance. This occurs when the tubercle (infected macrophage or granuloma) is not completely eradicated by the immune system. Therefore, the tubercles remain in the system for a longer period of time. This type of infection is called latent tuberculosis.

The latently infected person is non-infectious however the infection can be detected by tuberculin skin test (TST), and interferon gamma (IFN-γ) release assays (IGRA). While latent tuberculosis has a low potential to cause recurrent infection

(10%), it has been reported that reactivation of latent tuberculosis (active tuberculosis) may possibly happen after years of post-infection. The risk of reactivation is greater in HIV infected patients (15%) (Ahmad 2011). Studies have shown that, the pathogen enables the intracellular replication before responses of the immune system occurs in the lymph nodes and other extra pulmonary sites. This is an extraordinary ability that *M. tuberculosis* has to allow persistence and avoid eradication by host-immune system (Ahmad 2011, Obregon-Henao et al. 2012).

Unlike *M. tuberculosis*, non-tuberculous mycobacteria (NTM) are live in moist habitats such as lakes, rivers and damp-soil. For example, *M. avium* complex (MAC), *M. genavense*, *M. kansasii*, *M. xenopi*, *M. simiae*, *M. gordonae* and some RGM have been recovered from tap water (Han, De & Jacobson 2007, Esteban et al. 2008, Stout et al. 2011). Some NTM play important roles in nosocomial disease and pseudo-outbreaks. A recent study showed that NTM may be isolated from skin, upper respiratory tract, intestinal tract and genital tract and shows no symptoms to individuals (Lim, Kim & Yang 2012). Although the presence of NTM is not significant it indicates effects of infection and due to their ubiquity nature, their clinical significance is worth noting.



** TST and QuantiFERON-TB Gold test are commonly used for immunodiagnostic test.*

***Both immunodiagnostic and acid-fast bacilli (AFB) microscopy results analysis.*

Figure 1.4: The flow-chart shows natural cause progression of tuberculosis and general plan-of-detection in immunocompetent and immunocompromised individual following exposure to tubercle bacilli by inhalation.

1.4 Clinical significance.

1.4.1 Slowly-growing mycobacteria (SGM).

The higher prevalence of MTBC is known to involve several factors such as poverty, individual movement from high-risk countries, close-contact with infected tuberculosis patients and reactivation of latent tuberculosis among HIV infected persons (Pfyffer, Palicova 2011, Knechel 2009). MTBC consists of *M. tuberculosis*, *M. bovis*, *M. bovis* BCG (bacillus Calmette-Guérin), *M. africanum M. caprae*, *M. microti*, *M. canettii* and *M. pinnipedii*. They share genetic homology with no significant variation between sequences (~0.01 to 0.03%) although they have characterized difference with phenotypes (Homolka et al. 2008). Although all members of MTBC cause infection of humans, *M. bovis*, *M. bovis BCG*, *M. microti* and *M. pinnipedii* commonly infect warm-blooded animal as their primary host. It is likely that transmission occurs from animal to human (de la Rua-Domenech 2006, Leung et al. 2008, Kiers et al. 2008). Primarily, the animal to human tuberculosis transmission cases have been found from people who have worked or resided in the particular environment (Miltgen et al. 2002, Cvetnic et al. 2007, Romero et al. 2007, Kiers et al. 2008, Schoepf et al. 2012).

In adults, tuberculosis progression is slow. Tuberculosis infection shows several significant clinical manifestations in pulmonary and extrapulmonary sites (Knechel 2009). Prolonged coughing, severe weigh-lost, night sweats, low-grade fever, dyspnoea and chest pain are clinical symptoms indicated from pulmonary infections. The extrapulmonary manifestation of *M. tuberculosis* infection includes meningitis, pleuritis, pericarditis, synovitis, cervical lymphadenitis and infections of the skin, joints, bones and internal organs (Fitzgerald, Sterling & Haas 2010, Ahmad

2011). In HIV infected tuberculosis patients, both types of clinical manifestation are significant due to the rapid progression of infection.

M. africanum and *M. canetti* tuberculosis infection is mainly reported in tropical Africa (Miltgen et al. 2002, de Jong, Antonio & Gagneux 2010). It can be transmitted to western regions by migration of infected persons that used to live in Africa. Studies have shown that *M. africanum* can be subdivided into type I (*M. bovis*-like) and II (*M. tuberculosis*-like) based on chromosomal deletion distribution and geographic origin (Mostowy et al. 2004, de Jong, Antonio & Gagneux 2010). Infected patients have shown similar pulmonary clinical features regardless of type. Studies by de Jong et al. (2007) showed that a smear-positive tuberculosis patient identified various clinical and epidemiology differences between *M. africanum* type II and *M. tuberculosis*. Patients infected with this strain more likely showed major lung affects which were observed on chest X-rays regardless of a cough duration manifestation and occured in the group of older, HIV infected and malnourished patients (de Jong et al. 2007). *M. canetti* tuberculosis infection have also been highly reported in Africa with similar clinical features of the *M. africanum* infection. Although the main reservoir of *M. canetti* is unknown, patients show *M. tuberculosis* tuberculosis pulmonary clinical features and also lymphadenitis in tuberculosis infected children. Most of the cases have been reported from the continent of Africa (Miltgen et al. 2002).

1.4.2 Rapidly-growing mycobacteria (RGM).

The RGM are grouped as NTM and are opportunistic pathogens that can grow within 7 days in specific growth media. To date, more than 130 species of RGM have been found. Among these, several species have a clinical significance

and contribute to major health care-associated pseudo-outbreaks worldwide. Although many species have been identified as RGM, studies have shown that non-pigmented RGM, *M. fortuitum*, *M. chelonae* and *M. abscessus* are pathogenic and responsible for disease in humans (Han, De & Jacobson 2007, Chan et al. 2010).

Several cases have been reported due to the presence of RGM, commonly *M. fortuitum*, *M. chelonae* and *M. abscessus* and including less common (>10 clinical isolates or cases) human pathogenic RGM species; *M. phocacium, M. mucogenicum and M. smegmatis* group (known as sensu stricto). The common RGM species/groups cause health care-associated pseudo-outbreak diseases and are described in table 1.4. A study by Gayathri et al. (2010) has shown the *in vitro* antibiotic susceptibility testing among 148 RGM isolates and has indicated that a majority of RGM were sensitive to Amikacin (98%), Gatifloxacin (91%), Moxifloxacin (87%), Ciprofloxacin (76%) and Norfloxacin (Gayathri et al. 2010). Although the majority of health care-associated pseudo-outbreak diseases are treatable using antibiotics, the emergence of multidrug-resistant *M. abscessus* strains worsen the pseudo-outbreak infection scenario due to the most-virulent and chemotherapy-resistant strains (Leao et al. 2009, Gayathri et al. 2010).

Table 1.4: Most frequent human diseases caused by common RGM group/species (continued on next page).

Organism	Infection/disease	Clinical features	Potential host
M. fortuitum group	Post-traumatic wound	Localized cutaneous, localized cellulitis or abscesses	Normal or healthy patient undergone surgery
	open fracture following bone and join surgery,	Osteomyelitis, arthritis	Metal puncture wound and motor accident patient related cases
	Catheter infections	Bacteremia	Immunosuppressed patients receiving corticosteroid
	Post-keratoplasty and LASIK surgery	Keratitis	Normal or healthy patient undergone surgery
	Cosmetic/salon equipment contamination	Furunculosis	Normal or healthy patient exposed to contamination
M. chelonae	Post-surgical trauma	Desseminated cutaneous,	Immunosuppressed patients receiving corticosteroid
	Catheter infections	Hematogenous spread/bacteremia	Immunosuppressed patients receiving corticosteroid
	Chronic pulmonary infection	Lipoid pneumonia	Older non-smoking women on long-term corticosteroids therapy patient
	Post-keratoplasty and LASIK surgery	Keratitis	Normal or healthy patient undergone surgery

Table 1.4: Most frequent human diseases caused by common RGM group/species (continued on next page).

Organism	Infection/disease	Clinical features	Potential host
M. abscessus	Post-traumatic wound	Localized cutaneous	immunosuppressed patients receiving corticosteroid
	Post-surgical wound	Chronic disseminated cutaneous	Immunosuppressed patients
		Otitis	Ear tube placement surgical patients
	Pulmonary	Chronic lung infection	Cyctic fibrosis, *M. avium* complex infected patient, non-smoking older women with bronchiectasis. Chronically immunosuppressed patients receiving corticosteriod
	Catheter infections	Multiple draining cutaneous nodules at lower extremities	Immunosuppressed patients receiving corticosteroid
	Post-keratoplasty and LASIK surgery	Keratitis	Normal or healthy patient undergone surgery
M. smegmatis group	Post-surgical or medical procedures, face-lift plastic surgery	Cellulitis, localized abscess,	Normal or healthy patient undergone surgery
	Post-surgical open-fractures wound	Osteomyelitis	Normal or healthy patient undergone surgery

Table 1.4: Most frequent human diseases caused by common RGM group/species.

Organism	Infection/disease	Clinical features	Potential host
M. mucogenicum	Catheter infection	Bacteremia	Immunosuppressed patients receiving corticosteroids
M. fortuitum biovarint complex	Post-traumatic wound, Post-surgical open-fractures wound	Osteomyelitis	Metal puncture wound and motor accident patient related cases

LASIK= Laser-assisted in situ keratomileusis.

1.5 A standard diagnosis of *M. tuberculosis* from clinical specimens.

The most frequent type of sample in which the presence of *M. tuberculosis* is detected is respiratory expectorate or sputum. Samples are taken from potential patients after clinical manifestations are confirmed by chest X-rays, except for the HIV infected and elderly patients that do not show the typical pulmonary clinical features (Perkins, Cunningham 2007, Richter, Brown-Elliott & Wallace 2011). Conventionally, three sputum samples collected from persistent coughing patients are processed to ensure a large number of bacilli. Usually, there are a small numbers of bacilli present in body fluid samples. Therefore, the sample is suspended in sterile saline (0.85%) or bovine albumin (0.2%) and centrifuged (≥ 3,000 × g, 15 minutes) prior to inoculation of the sediment.

M. tuberculosis detection from pre-processed samples is performed by acid-fast staining to identify the presence of AFB. This is followed by culturing on solid media. Due to the slow-growing character of *M. tuberculosis*, at least 4 to 8 weeks are required for visible growth on solid media. The CDC has recommended that positive results from AFB smears must be reported after 24 hours of specimen receipt (Watterson, Drobniewski 2000, Knechel 2009). The standard culture media used in many diagnostic laboratories for identification of *M. tuberculosis* isolated from sputum samples is Löwenstein-Jensen (L~J) or Kirchner solid/liquid media and Middlebrook (7H9, 7H10 and 7H11) formulation. Following confirmation of tuberculosis from standard protocols, an AFB microscopy analysis must be performed to ensure the prescribed treatment is successful. However, it is unnecessary to obtain three samples to discontinue respiratory isolation.

References

Abe, C., Hosojima, S., Fukasawa, Y., Kazumi, Y., Takahashi, M., Hirano, K. & Mori, T. (1992) Comparison of MB-Check, BACTEC, and Egg-Based media for recovery of Mycobacteria. *Journal of Clinical Microbiology,* 30(4), 878-881.

Adekambi, T., Berger, P., Raoult, D. & Drancourt, M. (2006) *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. *International Journal of Systematic and Evolutionary Microbiology,* 56, 133-143.

Adekambi, T., Colson, P. & Drancourt, M. (2003) *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *Journal of Clinical Microbiology,* 41(12), 5699-5708.

Adekambi, T., Drancourt, M. & Raoult, D. (2008) The *rpo*B gene as a tool for clinical microbiologist. *Trends in Microbiology,* 17(1), 37-45.

Adékambi, T. & Drancourt, M. (2004) Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, *hsp65*, *sodA*, *recA* and *rpoB* gene sequencing. *International Journal of Systematic and Evolutionary Microbiology,* 54(6), 2095-2105.

Afonso, C. & Fenselau, C. (2003) Use of bioactive glass slides for matrix-assisted laser desorption/ionization analysis: Application to microorganisms. *Analytical Chemistry,* 75(3), 694-697.

Ahmad, S. (2011) Pathogenesis, immunology and diagnosis of latent *Mycobacterium tuberculosis* infection. *Clinical and Developmental Immunology,* 2011(814943).

Al-Mahruqi, S.H., van Ingen, J., Al-Busaidy, S., Boeree, M.J., Al-Zadjali, S., Patel, A., Dekhuijzen, P.N.R. & Soolingen, D.V. (2009) Clinical relevance of nontuberculous mycobacteria, Oman. *Emerging Infectious Diseases,* 15(2), 292-294.

Anderson, K.L. & Dunman, P.M. (2009) Messenger RNA turnover processes in *Escherichia coli*, *Bacillus subtilis* and emerging studies in *Staphylococcus aureus*. *International Journal of Microbiology,* 525491.

Anuchin, A.M., Mulyukin, A.L., Suzina, N.E., Duda, V.I., El-Registan, G.I. & Kaprelyants, A.S. (2009) Dormant forms of *Mycobacterium smegmatis* with distinct morphology. *Microbiology,* 155(4), 1071-1079.

Arend, S., Thijsen, S., Leyten, E., Bouwman, J., Willeke, P. & Franken, (2007) Comparison of two interferon-gamma and tuberculin skin test for tracing tuberculosi contacts. *American Journal of Respiratory and Critical Care Medicine,* 175, 618-27.

Aryan, E., Makvandi, M., Farajzadeh, A., Huygen, K., Bifani, P., Mousavi, S., Fateh, A., Jelodar, A., Gouya, M. & Romano, M. (2010) A novel and more sensitive loop-mediated isothermal amplification assay targeting IS*6110* for detection of *Mycobacterium tuberculosis* complex. *Microbiological Research*, 165(3), 211-220.

Balakrishnan, S., Vijayan, S., Nair, S., Subramoniapillai, J., Mrithyunjayan, S., Wilson, N., Satyanarayana, S., Dewan, P.K., Kumar, A.M.V., Karthickeyan, D., Willis, M., Harries, A.D. & Nair, S.A. (2012) High diabetes prevalence among tuberculosis cases in Kerala, India. *PLoS ONE,* 7(10), e46502.

Barnard, M., Heidi Albert, H., Gerrit Coetzee, G., O'Brien, R. & Bosman, M.E. (2008) Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. *American Journal of Respiratory and Critical Care Medicine*, 177, 787-792.

Barrett, A., Magee, J.G. & Freeman, R. (2002) An evaluation of the BD ProbeTec ET system for the direct detection of *Mycobacterium tuberculosis* in respiratory samples. *Journal of Medical Microbiology,* 51(10), 895-898.

Batz, H.G., Casenghi, M., Cooke, G.S., Hargreaves, S., Reid, S.D. & Syed, J. (2012) New research and development strategy for tuberculosis diagnostics urgently needed. *Lancet,* 12, 584-585.

Berney, M. & Cook, G.M. 2010, "Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia", *PloS ONE*, [Online], vol. 5, no. 1, pp. 7/1/2010-e8614. Available from: doi:10.1371/journal.pone.0008614. [11/7/2012].

Bhattacharya, S., Roy, R., Chowdhury, N.R., Dasgupta, A. & Dastidar, S.G. (2009) Comparison of novel bilayered medium with the conventional media for cultivation of *Mycobacterium tuberculosis*. *Indian Journal of Medical Research,* 130(Nov 2009), 561-566.

Billinger, M.E., Olivier, K.N., Viboud, C., de Oca, R.M., Steiner, C., Holland, S.M. & Prevots, D.R. (2009) Nontuberculous mycobacteria-associated lung disease in hospitalized persons, United States, 1998-2005. *Emerging Infectious Diseases,* 15(10), 1562-1569.

Binga, E.K., Lasken, R.S. & Neufeld, J.D. (2008) Something from (almost) nothing: the impact of multiple displacement amplification on microbial ecology. *The ISME Journal,* 2(3), 233-241.

Blanc, L., Falzon, D., Fitzpatrick, C., Floyd, K., Garcia, I., Glaziou, P., Gunneberg, C., Hiatt, T., Timimi, H., Uplekar, M. & Velebit, L. 2009, *Global tuberculosis control: A short update to the 2009 report*, World Health Organization Press, Geneva, Switzerland.

Bleck, C.K.E., Merz, A., Gutierrez, M.G., Walther, P., Dubochet, J., Zuber, B. & Griffiths, G. (2009) Comparison of different methods for thin section EM analysis of *Mycobacterium smegmatis*. *Journal of Microscopy,* 237(1), 23-38.

Brennan, P.J. (2003) Structure, function and biogenesis of the cell wall of *Mycobacterium tuberculosis*. *Tuberculosis,* 83, 91-97.

Brown-Elliott, B.A. & Wallace, R.J.J. 2011, "*Mycobacterium*: Clinical and laboratory characteristics of rapidly growing mycobacteria" in *Manual of Clinical Microbiology Volume 1*, eds. J. Versalovic, K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry & D.W. Warnock, 10th edn, ASM Press, Washington DC, pp. 525-538.

Brown-Elliott, B.A. & Wallace, R.J., Jr. (2002) Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clinical Microbiology Reviews,* 15(4), 716-746.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J. & Wittwer, C.T. (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry,* 55(4), 611-622.

Caminero,J.A. (2006) Treatment of multidrug-resistant tuberculosis: Evidence and controversies. *International Journal of Tuberculosis and Lung Diseases*, 10(8), 829-837.

Cappucino, J.G. & Sherman, N. (1998) *Microbiology A Laboratory Manual.* 5th edn, California: Benjamin/Cummings Science Publishing.

Carson, S., Miller, H.B. & Witherow, D.S. 2012, "Lab Session 15 - Total RNA Purification" in *Molecular Biology Techniques (Third Edition)* Academic Press, San Diego, pp. 125-132.

Casabona, N.M., Bahr, A.R., Bennedsen, J., Thomsen, V.O., Curcio, M., Dufaux, M.F., Feldmen, K., Havelkova, M., Katila, M.L., Koksalan, K., Pereira, M.F., Rodrigues, F., Pfyffer, G.E., Portaels, F., Urgell, J.R., Gerdes, S.R., Spanish Group for Non-Tuberculosis Mycobacteria., Tortoli, E., Vincent, V. & Watt, B. (2004) Non-tuberculous mycobacteria: Patterns of isolation. A multi-country retrospective survey. *International Journal of Tuberculosis and Lung Diseases*, 8(10), 1186-1193.

Cauwelaert, N.D., Ramarokoto, H., Ravololonandriana, P., Richard, V. & Rasolofo, V. (2011) DNA extracted from stained sputum smears can be used in the MTBDR *plus* assay. *Journal of Clinical Microbiology,* 49(10), 3600-3603.

Cavalcanti, H. R., E. Marques, L. de Sauza Fonseca, and M. H. Feres Saad. 2007. Do DNA extraction methods and *Taq* polymerase quality improve the double

repetitive (DRE) PCR typing method for *Mycobacterium tuberculosis* strain. *Brazillian Journal of Microbiology*, 38**,** 409-412.

Caviedes, L., Lee, T., Gilman, R.H., Sheen, P., Spellman, E., Lee, E.H., Berg, D.E., Montenegro-James, S. & The Tuberculosis Working Group in Peru (2000) Rapid, efficient detection and drug susceptibility testing of *Mycobacterium tuberculosis* in sputum by microscopic observation of broth cultures. *Journal of Clinical Microbiology,* 38(3), 1203-1208.

Chacon, O., Feng, Z., Harris, N.B., Caceres, N.E., Adams, L.G. & Barletta, R.G. (2002) *Mycobacterium smegmatis* D-alanine racemase mutants are not dependent on D-alanine for growth. *Antimicrobial Agents and Chemotherapy,* 46(1), 47-54.

Chan, E.D., Bai, X., Kartalija, M., Orme, I.M. & Ordway, D.J. (2010) Host immune response to rapidly growing mycobacteria, an emerging cause of chronic lung disease. *American Journal of Respiratory Cell and Molecular Biology,* 43(4), 387-393.

Chang, S., Rihana, A., Bahrman, S., Gruden, C.L., Khijniak, A.I., Skerlos, S.J. & Adriaens, P. (2004) Flow cytometric detection and quantification of mycobacteria in metalworking fluids. *International Biodeterioration & Biodegradation*, 54(2–3), 105-112.

Chomczynski, P. & Sacchi, N. (2006) The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. *Nature Protocol,* 1(2), 581-585.

Clement-Ziza, M., Munnich, A., Lyonnet, S., Jaubert, F. & Besmond, C. (2008) Stabilization of RNA during laser capture microsdissection by perfoming experiments under argon atmosphere or using ethanol as a solvent in staining solutions. *RNA*, 14, 2698-2704.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E., Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T.,

Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M.A., Rajandream, M.A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J.E., Taylor, K., Whitehead, S. & Barrel, B.G. (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature,* 393, 537-544.

Connell, T., Ritz, N., Paxton, G., Buttery, J., Curtis, N. & Ranganathan, S. (2008) A three-way comparison of tuberculin skin testing, QuantiFERON-TB Gold and T-SPOT. TB in children. *PLoS ONE,* 3(7), e2624.

Cornea, A. & Mungenast, C. (2002) Comparison of current equipment. *Methods in Enzymology,* 356(Laser Capture Microscope), 3.

Csanadi, A., Faludi, I. & Miczak, A. (2009) MSMEG_4626 ribonuclease from *Mycobacterium smegmatis*. *Molecular Biology Reports,* 36, 2341-2344.

Curran, S., McKay, J.A., McLeod, H.L. & Murray, G.I. (2000) Laser capture microscopy. *Molecular Pathology,* 53, 64-68.

Cvetnic, Z., Jankovic, K., Sostaric, B., Spicic, S., Obrovac, M., Marjanovic, S., Benic, M., Kirin, B.K. & Vickovic, I. (2007) *Mycobacterium caprae* in cattle and humans in Croatia. *International Journal of Tuberculosis and Lung Disease,* 11(6), 652-658.

Dagnew, A., Hussein, J., Abebe, M., Zewdie, M., Mihret, A., Bedru, A., Chanyalew, M., Yamuah, L., Medhin, G., Bang, P., Doherty, T., Hailu, A. & Aseffa, A. (2012) Diagnosis of latent tuberculosis infection in healthy young adults in a country with high tuberculosis burden and BCG vaccination at birth. *BMC Research Notes,* 5(1), 415.

Davey, H.M. & Winson, M.K. (2003) Using Flow cytometry to quantify microbial heterogeneity. *Current Issues in Molecular Biology.,* 5, 9-15.

de Jong, B.C., Hill, P.C., Aiken, A., Jeffries, D.J., Onipede, A., Small, P.M., Adegbola, R.A. & Corrah, T.P. (2007) Clinical presentation and outcome of

tuberculosis patients infected by *M. africanum* versus *M. tuberculosis*. *International Journal of Tuberculosis and Lung Disease,* 11(4), 450-456.

de Jong, B.C., Antonio, M. & Gagneux, S. (2010) *Mycobacterium africanum*—Review of an important cause of human tuberculosis in West Africa. *PLoS Neglected Tropical Diseases,* 4(9), e744.

de la Rua-Domenech, R. (2006) Human *Mycobacterium bovis* infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. *Tuberculosis (Edinburgh, Scotland),* 86(2), 77-109.

Dean, F.B., Hosono, S., Fang, L., Wu, X., Faruqi, A.F., Bray-Ward, P., Sun, Z., Zong, Q., Du, Y.D.,J., Driscoll, M., Song, W., Kingsmore, S.F., Egholm, M. & Lasken, R.S. (2002) Comprehensive human genome amplification using multiple displacement amplification. *PNAS,* 99(8), 5261-5266.

Dias, H.M., Falzon, D., Fitzpatrick, C., Floyd, K., Glaziou, P., Hiatt, T., Lienhardt, C., Nguyen, L., Sismanidis, C., Timimi, H., Uplekar, M., van Gemert, W. & Zignol, M. 2012, *Global Tuberculosis Report 2012*. World Health Organization, Geneva,Switzerland.

Diaza, R., Siddiqi, N. & Rubin, E.J. (2006) Detecting genetic variability among different *Mycobacterium tuberculosis* strains using DNA microarrays technology. *Tuberculosis,* (86), 314-318.

Dick, T., Boon, H.L. & Murugasu-Oei, B. (1998) Oxygen depletion induced dormancy in *Mycobacterium smegmatis*. *FEMS Microbiology Letters,* 163(2), 159-164.

Douglas W. Selinger, D.W., Rini Mukherjee Saxena, R.M., Kevin J. Cheung, K.J., Church, G.M. & Rosenow, C. 2003, "Global RNA half-life analysis in *Escherichia coli* reveals positional patterns of transcript degradation", *Genome Research,* [Online], pp. 28.11.2012. Available from: http://www.genome.org/cgi/doi/10.1101/ gr.912603.

Drake, W.P., Pei, Z., Pride, D.T., Collins, R.D., Cover, T.L. & Blaser, M.J. (2002) Molecular analysis of sarcoidosis tissues for *Mycobacterium* species DNA. *Emerging Infectious Diseases,* 8(11), 1334-1341.

Draper, P. (1998) The outer parts of the mycobacterial envelope as permeability barriers. *Frontiers in Bioscience,* 3, 1253-1261.

Duthie, S., Black, C., Douglas, G., Jackson, A.D. & Webster, D. (2008) Tuberculosis outbreak associated with a mosque: Challenges of large scale contact tracing. *Eurosurveillance,* 13(51), pii=19069.

Eid,A.J., Berbari, E.F., Sia, I.G., Wengenack, N.L., Osmon, D.R. & Razonable, R.R. (2007) Prosthetic joint infection due to rapidly growing mycobacteria: Report of 8 cases and review of the literature. *Clinical Infectious Diseases,* 45(6), 687-697.

El Amin, N.M., Hanson, H.S., Pettersson, B., Petrini, B. & Stedingk, L.V.V. (2000) Identification of non-tuberculous mycobacteria: 16S rRNA gene sequence analysis vs. conventional methods. *Scandinavian Journal of Infectious Diseases,* 32(1), 47-50.

El Khechine, A., Henry, M., Raoult, D. & Drancourt, M. (2009) Detection of *Mycobacterium tuberculosis* complex organisms in the stools of patients with pulmonary tuberculosis. *Microbiology,* 155(7), 2384-2389.

Estaban, J.A., Salas, M. & Blanco, L. (1993) Fidelity of Φ DNA polymerase. comparison between protein-primed initiation and DNA polymerization. *Journal of Biological Chemistry,* 268(4), 2719-2726.

Esteban, J., Martín-de-Hijas, N.Z., Fernandez, A.I., Fernandez-Roblas, R. & Gadea, I. (2008) Epidemiology of infections due to nonpigmented rapidly growing mycobacteria diagnosed in an urban area. *European Journal of Clinical Microbiology Infectious Diseases,* (27), 951-957.

Fang, Z., Doig, C., Kenna, D.T., Smittipat, N., Palittapongarnpim, P., Watt, B. & Forbes, K.J. (1999) IS*6110*-mediated deletions of wild-type chromosomes of *Mycobacterium tuberculosis*. *Journal of Bacteriology,* 181(3), 1014-1020.

Figueroa-Munoz, J., Floyd, K., Getahun, H., Jaramillo, E., Maher, D., Weil, D. & Williams, B. 2006, 2012-last update, *The stop TB strategy.* [Homepage of World Health Organization], [Online]. Available: http://www.who.int/tb/strategy/en/ [2012, 29.6.2012].

Fitzgerald, D.W., Sterling, T.R. & Haas, D.W. 2010, "*Mycobacterium tuberculosis*" in *Principles and Practice of Infectious Diseases.*, eds. G.L. Mandel, J.E. Bennett & R. Dolin, 7th edn, Churchill Livingstone Elsevier, Philadelphia, pp. 3129-3163.

Fitzpatrick, C., Floyd, K., Lienhardt, C. & Mandelbaum-Schmid, J. 2012, *The Global Plan to Stop TB*, World Health Organization, Geneva.

Foongladda, S., Pholwat, S., Eampokalap, B., Kiratisin, P. & Sutthent, R. (2009) Multi-probe real-time PCR identification of common *Mycobacterium* species in blood culture broth. *The Journal of Molecular Diagnostics* 11(1), 42-48.

Forbes, B.A. 2011, "Molecular detection and characterization of *Mycobacterium tuberculosis*" in *Molecular microbiology: Diagnostic principles and practice*, eds. D.H. Persing, F.C. Tenover, Y.J. Tang, F.S. Nolte, R.T. Hayden & A.V. Belkum, 2nd edn, ASM Press, Washington,DC, pp. 415-436.

Ford, J.G., Huang, A.J., Pflugfelder, S.C., Alfonso, E.C., Forster, R.K. & Miller, D. (1998) Nontuberculous mycobacterial keratitis in South Florida. *Ophthalmology*, 105(9), 1652-1658.

Fukunaga, H., Murakami, T., Gondo, T.S.,K. & Ishihara, T. (2002) Sensitivity of acid-fast staining for *Mycobacterium tuberculosis* in formalin-fixed tissue. *American Journal of Respiratory and Critical Care Medicine,* 166, 994-997.

Gadagkar, R. & Gopinathan, K.P. (1980) Growth of *Mycobacterium smegmatis* in minimal and complete media. *Journal of Biosciences,* 2(4), 337-348.

Gayathri, R., Therese, K., Deepa, P., Mangai, S. & Madhavan, H. (2010) Antibiotic susceptibility pattern of rapidly growing mycobacteria. *Journal of Postgraduate Medicine,* 56(2), 76-78.

Giardina, E., Pietrangeli, I., Martone, C., Zampatti, S., Marsala, P., Gabriele, L., Ricci, O., Solla, G., Asili, P., Arcudi.G., Spinella, A. & Novelli, G. (2009) Whole genome amplification and real-time PCR in forensic casework. *BMC Genomics,* 10, 159.

Gingeras, T.R., Ghandour, G., Wang, E., Berno, A., Small, P.M., Drobnieski, F., Alland, D., Desmond, E., Holodniy, M. & Drenkow, J. (1998) Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic *Mycobacterium* DNA arrays. *Genome Research,* 8, 435-448.

Gonzalez, J.M., Portillo, M.C. & Saiz-Jimenez, C. (2005) Multiple displacement amplification as a pre-polymerase chain reaction (pre-PCR) to process dificult to amplify samples and low copy number sequnces from natural environments. *Environmental Microbiology*, 7(7), 1024-1028.

Gordon, R.E. & Smith, M.M. (1955) Rapidly growing, acid fast bacteria. *Journal of Bacteriology*, 69(5), 502-507.

Gordon, R.E. & Smith, M.M. (1953) Rapidly growing, acid past bacteria: Species descriptions of *Mycobacterium Phlei* Lehmann and Neumann and *Mycobacterium smegmatis* (Trevisan) Lehmann and Neumann. *Journal of Bacteriology*, 66(1), 41-48.

Grover, A., Azmi, W., Paul Khurana, S.M. & Chakrabarti, S.K. (2009) Multiple displacement amplification as a pre-polymerase chain reaction (Pre-PCR) to detect ultra low population of Ralstonia solanacearum (Smith 1896) Yabuchi et al. (1996). *Letters in Applied Microbiology,* 49, 539-543.

Hahn, S., Zhong, X.Y. & Holzgreve, W. (2002) Single cell PCR in laser capture microscope. *Methods in Enzymology,* 356(Laser Capture Microscope), 295.

Halse, T.A., Edwards, J., Cunningham, P.L., Wolfgang, W.J., Dumas, N.B., Escuyer, V.E. & Musser, K.A. (2010) Combined real-time PCR and *rpoB* gene pyrosequencing for rapid identification of *Mycobacterium tuberculosis* and determination of rifampin resistance directly in clinical specimens. *Journal of Clinical Microbiology,* 48(4), 1182-1188.

Han, X.Y., De, I. & Jacobson, K.L. (2007) Rapidly growing mycobacteria: Clinical and microbiologic studies of 115 cases. *American Journal of Clinical Pathology,* 128, 612-621.

Helb, D., Jones, M., Story, E., Boehme, C., Wallace, E., Ho, K., Kop, J., Owens, M.R., Rodgers, R., Banada, P., Safi, H., Blakemore, R., Lan, N.T.N., Jones-Lopez, E.C., Levi, M., Burday, M., Ayakaka, I., Mugerwa, R.D., McMillan, B., Winn-Deen, E., Christel, L., Dailey, P., Perkins, M.D., Persing, D.H. & Alland, D. (2010) Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology,* 48(1), 229-237.

Henry, D.A. & Spreet, D.P. 2011, "*Pseudomonas*" in *Manual of Clinical Microbiology.*, eds. J. Versalovic, K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry & D.W. Warnock, 10th edn, ASM Press, Washington DC, pp. 677-691.

Hetherington, S.V., Watson, A.S. & Patrick, C.C. (1995) Sequence and analysis of the *rpoB* gene of *Mycobacterium smegmatis. Antimicrobial Agents and Chemotherapy,* 39(9), 2164-2166.

Hett, E.C. & Rubin, E.J. (2008) Bacterial growth and cell division:a Mycobacterial perspective. *Microbiology and Molecular Biology Reviews,* 72(1), 126-156.

Hofmann-Thiel, S., Turaev, L. & Hoffmann, H. (2010) Evaluation of the hyplex® TBC PCR test for detection of *Mycobacterium tuberculosis* complex in clinical samples. *BMC Microbiology,* 10, 95.

Homolka, S., Post, E., Oberhauser, B., George, A., Westman, L., Dafae, F., Rusch-Gerdes, S. & Niemann, S. (2008) High genetic diversity among *Mycobacterium tuberculosis* complex strains from Sierra Leone. *BMC Microbiology,* 8(1), 103.

Hosono, S., Faruqi, A.F., Dean, F.B., Du, Y., Sun, Z., Wu, X., Du, J., Kingsmore, S.F., Egholm, M. & Lasken, R.S. (2003) Unbiased whole-genome amplification directly from clinical samples. *Genome Research,* 13(5), 954-964.

Ikegame, S., Sakoda, Y., Fujino, N., Taguchi, K., Kawasaki, M. & Kajiki, A. (2012) Clinical Evaluation of COBAS *Taq*Man PCR for the Detection of *Mycobacterium tuberculosis* and *M. avium* complex. *Tuberculosis Research and Treatment,* 2012, 170459.

Ishoey, T., Woyke, T., Stepanauskas, R., Novotny, M. & Lasken, R.S. (2008) Genomic sequencing of single microbial cells from environmental samples. *Current Opinion in Microbiology,* 11(3), 198-204.

Jahn, C.E., Charkowski, A.O. & Willis, D.K. (2008) Evaluation of isolation methods and RNA integrity for bacterial RNA quantitation. *Journal of Microbiological Methods,* 75, 318-324.

Kamble, R.R., Shinde, V.S., Madhale, S.P., Kamble, A.A., Ravikumar, B.P. & Jadhav, R.S. (2010) Extraction and detection of *Mycobacterium leprae* DNA from ZNCF-stained skin smear slides for better identification of negative skin smears. *Indian Journal of Medical Microbiology,* 28(1), 57-59.

Kamerbeek, J., Schouls, L., Kolk, A., Agterveld, M.V., Soolingen, D.V., Kuijper, S., Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M. & Embden, J.V. (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *Journal of Clinical Microbiology,* 35(4), 907-914.

Kiers, A., Klarenbeek, A., Mendelts, B., van Soolingen, D. & Koëter, G. (2008) Transmission of *Mycobacterium pinnipedii* to humans in a zoo with marine mammals. *International Journal of Tuberculosis and Lung Disease,* 12, 1469-1473.

Kim, E.Y., Nahid, P., Hopewell, P.C. & Kato-Maeda, M. (2010) Novel hot spot of IS*6110* insertion in *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology,* 48(4), 1422-1424.

Kim, J.U., Cha, C.H. & An, H.K. (2012) Multiplex real-time PCR assay and melting curve analysis for identifying *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria. *Journal of Clinical Microbiology,* 50(2), 483-487.

Klitgaard, K., Jensen, T.K., Angen, O. & Boye, M. (2007) Measurement of bacterial gene expression in vivo by laser capture microdissection and quantitative real-time RT-PCR. *Journal of Microbiological Methods,* 69, 414-416.

Klitgaard, K., Molbak, L., Jensen, T.K., Lindboe, C.F. & Boye, M. (2005) Laser capture microdissection of bacterial cells targeted by fluorescence in situ hybridization. *BioTechniques,* 39(6), 864-868.

Knechel, N.A. (2009) Tuberculosis: Pathophysiology, clinical features, and diagnosis. *Critical Care Nurse,* 29(2), 34-43.

Kocagoz, T., Saribas, Z. & Alp, A. (2005) Rapid determination of rifampin resistance in clinical isolates of *Mycobacterium tuberculosis* by real-time PCR. *Journal of Clinical Microbiology,* 43(12), 6015-6019.

Kolbert, C.P. & Persing, D.H. (1999) Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Current Opinion in Microbiology,* 2(3), 299-305.

Kolk, A.H.J., Noordhoek, G.T., Leeuw, O.D., Kuijper, S. & Embden, V.D.A. (1994) *Mycobacterium smegmatis* strain for detection of *Mycobacterium tuberculosis* by PCR used as internal control for inhibition of amplification and for quantification of bacteria. *Journal of Clinical Microbiology,* 32(5), 1354-1356.

Lasken, R.S. & Egholm, M. (2003) Whole genome amplification:abundant supplies of DNA from precious samples or clinical specimens. *TRENDS in Biotechnology,* 21(12), 531-535.

Lasken, R.S. (2007) Single-cell genomic sequencing using multiple displacement amplification. *Current Opinion in Microbiology,* 10(5), 510-516.

Leao, S.C., Tortoli, E., Vianna-Niero, C., Ueki, S.Y.M., Lima, K.V.B., Lopez, M.L., Yubero, J., Menendez, M.C. & Garcia, M.J. (2009) Characterization of mycobacteria from a major Brazilian outbreak suggests that revision of the taxanomic status of members of *Mycobacterium chelone-M. abscessus* group is needed. *Journal of Clinical Microbiology*, 47(9), 2691-2698.

Lebrun, L., Weill, F., Lafendi, L., Houriez, F., Casanova, F., Gutierrez, M.C., Ingrand, D., Lagrange, P., Vincent, V. & Herrmann, J.L. (2005) Use of the INNO-LiPA-MYCOBACTERIA Assay (Version 2) for identification of *Mycobacterium avium- Mycobacterium intracellulare -Mycobacterium scrofulaceum* Complex Isolates. *Journal of Clinical Microbiology,* 43(6), 2567-2574.

Lee, H., Bang, H.E., Bai, G.H. & Cho, S.N. (2003) Novel polymorphic region of the *rpoB* gene containing *Mycobacterium* species-specific sequences and its use in identification of Mycobacteria. *Journal of Clinical Microbiology,* 41(5), 2213-2218.

Lee, J.Y., Choi, H.J., Park, I., Hong, S., Oh, Y. & Lim, C. (2006) Comparison of two commercial interferon-gamma assays for diagnosing *Mycobacterium tuberculosis* infection. *European Respiratory Journal,* 28, 24-30.

Lessells, R.J., Cooke, G.S., Newell, M. & Godfrey-Faussett, P. (2011) Evaluation of tuberculosis diagnostics: Establishing an evidence base around the public health impact. *Journal of Infectious Diseases,* 204(suppl 4), S1187-S1195.

Leung, A., Tran, V., Wu, Z., Yu, X., Alexander, D., Gao, G., Zhu, B. & Liu, J. (2008) Novel genome polymorphisms in BCG vaccine strains and impact on efficacy. *BMC Genomics,* 9(1), 413.

Lewin, A., Freytag, B., Meister, B., Sharbati-Tehrani, S., Schäfer, H. & Appel, B. (2003) Use of a Quantitative *Taq*Man-PCR for the fast quantification of mycobacteria in broth culture, eukaryotic cell culture and tissue. *Journal of Veterinary Medicine, Series B,* 50(10), 505-509.

Lim, J.M., Kim, J.H. & Yang, H.J. (2012) Management of infections with rapidly growing mycobacteria after unexpected complications of skin and subcutaneous surgical procedures. *Archives of Plastic Surgery*, 39(1), 18-24.

Long, Q., Zhou, Q., Ji, L., Wu, J., Wang, W. & Xie, J. (2012) *Mycobacterium smegmatis* genomic characteristics associated with its saprophyte lifestyle. *Journal of Cellular Biochemistry,* 113(10), 3051-3055.

Mangan, J.A., Sole, K.M., Mitchison, D.A. & Butcher, P.D. (1997) An effective method of RNA extraction from bacteria refractory to disruption, including mycobacteria. *Nucleic Acids Research,* 25(3), 675-676.

Martinez, A.N., Lahiri, R., Pittman, T.L., Scollard, D., Truman, R., Moraes, M.O. & Williams, D.L. (2009) Molecular determination of *Mycobacterium leprae v*iability by use of real-time PCR. *Journal of Clinical Microbiology,* 47(7), 2124-2130.

Mase, S.R., Ramsay, A., Ng, V., Henry, M., Hopewell, P.C., Cunningham, J., Urbanczik, R., Perkins, M.D., Aziz, M.A. & Pai, M. (2007) Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: A systematic review. *The international journal of tuberculosis and lung disease: The official journal of the International Union against Tuberculosis and Lung Disease,* 11(5), 485-495.

Mazurek, G.H., Jereb, J., Lobue, P., Iademarco, M.F., Metchock, B., Vernon, A. & Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention (2005) Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recommendations and Reports,* 54, 49-55.

McNerney, R. Wondafrash, B., Amena, K., Tesfaye, A., McCash, E. & Murray, N. (2010) Field test of novel detection device for *Mycobacterium tuberculosis* antigen in cough. *BMC Infectious Diseases*, 10(1), 161.

Medjahed, H., Gaillard, J. & Reyrat, J. (2010) *Mycobacterium abscessus*: a new player in the mycobacterial field. *Trends in microbiology,* 18(3), 117-123.

Menzies, D., Pai, M. & Comstock, G. (2007) Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Annals of Internal Medicine,* 14, 340-54.

Meyers, P.R., Bourn, W.R., Steyn, L.M., van Helden, P.D., Beyers, A.D. & Brown, G.D. (1998) Novel method for rapid measurement of growth of mycobacteria in detergent-free media. *Journal of Clinical Microbiology,* 36(9), 2752-2754.

Miltgen, J., Morillon, M., Koeck, J.L., Varnerot, A., Briant, J.F., Nguyen, G., Verrot, D., Bonnet, D. & Vincent, V. (2002) Two cases of pulmonary tuberculosis caused by *Mycobacterium tuberculosis subsp. canetti*. *Emerging Infectious Diseases,* 8(11), 1350-1352.

Minion, J., Leung, E., Menzies, D. & Pai, M. (2010) Microscopic-observation drug susceptibility and thin layer agar assays for the detection of drug resistant tuberculosis: a systematic review and meta-analysis. *The Lancet Infectious Diseases,* 10(10), 688-698.

Monger, B.C. & Landry, M.R. (1993) Flow cytometric analysis of marine bacteria with Hoechst 33342. *Applied and Environmental Microbiology,* 59, 905-911.

Morgan, M., Kalantri, S., Flores, L. & Pai, M. (2005) A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: A systematic review and meta-analysis. *BMC Infectious Diseases,* 5, 62.

Mostowy, S., Onipede, A., Gagneux, S., Niemann, S., Kremer, K., Desmond, E.P., Maeda, M.K. & Behr, M. (2004) Genomic analysis distinguishes *Mycobacterium africanum*. *Journal of Clinical Microbiology,* 42(8), 3594-3599.

Nebe-von-Caron, G. (2009) Standardization in microbial cytometry. *Cytometry Part A,* 75(A), 86-89.

Nebe-von-Caron, G., Stephens, P.J., Hewitt, C.J., Powell, J.R. & Badley, R.A. (2000) Analysis of bacterial function by multi-colour fluorescence flow cytometry and single cell sorting. *Journal of Microbiological Methods,* 42, 97-114.

Ninet, B., Monod, M., Emler, S., Pawlowski, J., Metral, C., Rohner, P., Auckenthaler, R. & Hirschel, B. (1996) Two different 16S rRNA genes in a mycobacterial strain. *Journal of Clinical Microbiology,* 34(10), 2531-2536.

Noens, E., Williams, C., Anandhakrishnan, M., Poulsen, C., Ehebauer, M. & Wilmanns, M. (2011) Improved mycobacterial protein production using a *Mycobacterium smegmatis* groEL1DeltaC expression strain. *BMC Biotechnology,* 11(1), 27.

Noordhoek, G.T., Embden van, J.D.A. & Kolk, A.H.J. (1996) Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*:An international collaborative quality control study among 30 laboratories. *Journal of Clinical Microbiology,* 34(10), 2522-2525.

Obregon-Henao, A., Duque-Correa, M.A., Rojas, M., Garcia, L.F., Brennan, P.J., Ortiz, B.L. & Belisle, J.T. (2012) Stable extracellular RNA fragments of Mycobacterium tuberculosis induce early apoptosis in human monocytes via a caspase-8 dependent mechanism. *PloS ONE,* 7(1), e29970.

Ong, D.C.T., Yam, W.C., Siu, G.K.H. & Lee, A.S.G. (2010) Rapid detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* by high-resolution melting analysis. *Journal of Clinical Microbiology,* 48(4), 1047-1054.

Oto, M., Suda, W. & Shinoyama, H. (2006) 16S rRNA Gene-based analysis of microbial community by whole-genome amplification and minigel-single-strand conformation polymorphism technique. *Journal of Bioscience and Bioengineering,* 102(5), 482-484.

Pai, M., Kalantri, S. & Dheda, K. (2006) New tools and emerging technologies for the diagnosis of tuberculosis: Part II. Active tuberculosis and drug resistance. *Expert Review of Molecular Diagnostics,* 6(3), 423-432.

Pandey, A., Madan, M., Asthana, A.K., Kansal, R. & Das, A. (2009) Cold acid fast staining method: Efficacy in diagnosis of *Mycobacterium tuberculosis*. *African Journal of Microbiology Research,* 3(9), 546-549.

Park, H., Jang, H., Kim, C., Chung, B., Chang, C.L., Park, S.K. & Song, S. (2000) Detection and identification of mycobacteria by amplification of the internal transcribed spacer regions with genus- and species-specific PCR primers. *Journal of Clinical Microbiology,* 38(11), 4080-4085.

Patnaik, M., Liegmann, K. & Peter, J.B. (2001) Rapid detection of smear-negative *Mycobacterium tuberculosis* by PCR and sequencing for rifampin resistance with DNA extracted directly from slides. *Journal of Clinical Microbiology,* 39(1), 51-52.

Perkins, M.D. & Cunningham, J. (2007) Facing the crisis: Improving the diagnosis of tuberculosis in the HIV era. *Journal of Infectious Diseases,* 196, 15-27.

Peter-Getzlaff, S., Luthy, J., Boddinghaus, B., Bottger, E.C. & Springer, B. (2008) Development and evaluation of a molecular assay for detection of nontuberculous mycobacteria by use of the cobas amplicor platform. *Journal of Clinical Microbiology,* 46(12), 4023-4028.

Peter-Getzlaff, S., Luthy, J., Voit, A., Bloemberg, G.V. & Bottger, E.C. (2010) Detection and identification of *Mycobacterium* spp. in clinical specimens by combining the Roche Cobas Amplicor *Mycobacterium tuberculosis* assay with Mycobacterium genus detection and nucleic acid sequencing. *Journal of Clinical Microbiology,* 48(11), 3943-3948.

Petti, C.A., Weinstein, M.P. & Carroll, K.C. 2011, "Systems for Detection and Identification of Bacteria and Yeast." in , eds. J. Versalovic, K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry & D.W. Warnock, 10th edn, ASM Press, Washington DC, pp. 15-26.

Pfyffer, G.E. & Palicova, F. 2011, "*Mycobacterium*: General Characteristics, Laboratory Detection, and Staining Procedures." in *Manual of Clinical Microbiology. Volume 1.*, eds. J. Versalovic, K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry & D.W. Warnock, 10th edn, ASM Press, Washington, DC, pp. 472-502.

Phetsuksiri, B., Rudeeaneksin, J., Supapkul, P., Wachapong, S., Mahotarn, K. & Brennan, P.J. (2006) A simplified reverse transcriptase PCR for rapid detection of *Mycobacterium leprae* in skin specimens. *FEMS Immunology & Medical Microbiology,* 48(3), 319-328.

Radstrom, P., Knutsson, R., Wolffs, P.L.,M. & Lofstrom, C. (2004) Pre-PCR processing. *Molecular Biotechnology*, 26, 133-146.

Raghunathan, A., Ferguson, H.R., Bornarth, C.J., Song, W., Driscoll, M. & Lasken, R.S. (2005) Genomic DNA amplification from a single bacterium. *Applied and Environmental Microbiology,* 71(6), 3342-3347.

Rao, V., Fujiwara, N., Porcelli, S.A. & Glickman, M.S. (2005) *Mycobacterium tuberculosis* controls host innate immune activation through cyclopropane modification of a glycolipid effector molecule. *The Journal of Experimental Medicine*, 201(4), 535-543.

Reddington, K., O'Grady, J., Dorai-Raj, S., Maher, M., van Soolingen, D. & Barry, T. (2011a) Novel multiplex real-time PCR diagnostic assay for identification and differentiation of *Mycobacterium tuberculosis*, *Mycobacterium canettii*, and *Mycobacterium tuberculosis* complex strains. *Journal of Clinical Microbiology*, 49(2), 651-657.

Reddington, K., O'Grady, J., Dorai-Raj, S., Niemann, S., van Soolingen, D. & Barry, T. (2011b) A Novel multiplex real-time PCR for the identification of mycobacteria associated with zoonotic tuberculosis. *PLoS ONE,* 6(8), e23481.

Reddy, S., Brown, T. & Drobniewski, F. (2010) Detection of *Mycobacterium tuberculosis* from paraffin-embedded tissues by INNO-LiPA Rif.TB assay:

retrospective analyses of Health Protection Agency National *Mycobacterium* Reference Laboratory data. *Journal of Medical Microbiology*, 59(5), 563-566.

Richter, E., Brown-Elliott, B.A. & Wallace, R.J.J. 2011, "*Mycobacterium*:Laboratory Characteristics of Slowly Growing Mycobacteria." in *Manual of Clinical Microbiology*, eds. J. Versalovic, K.C. Carroll, G. Funke, J.H. Jorgensen, M.L. Landry & D.W. Warnock, 10th edn, ASM Press, Washington DC, pp. 503-524.

Romero, B., Aranaz, A., Bezos, J., Álvarez, J., de Juan, L., Tariq Javed, M., Mateos, A., Gómez-Mampaso, E. & Domínguez, L. (2007) Drug susceptibility of Spanish *Mycobacterium tuberculosis* complex isolates from animals. *Tuberculosis*, 87(6), 565-571.

Rusch-Gerdes, S. & Richter, E. (2004) Clinical evaluation of the semiautomated BDProbeTec ET System for the detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. *Diagnostic Microbiology and Infectious disease,* 48(4), 265-270.

Rustad, T.R., Minch, K.J., Brabant, W., Winkler, J.K., Reiss, D.J., Baliga, N.S. & Sherman, D.R. (2012) Global analysis of mRNA stability in *Mycobacterium tuberculosis*. *Nucleic acids research,* 2012, 1-9.

Ryan, P., Bennet, M.W., Aarons, S., Lee, G., Collins, J.K., O'Sullivan, G.C., O'Connell, J. & Shanahan, F. (2002) PCR detection of *Mycobacterium paratuberculosis* in Crohn's disease granulomas isolated by laser capture microdissection. *Gut,* 51, 665-670.

Sanjeev, H., Karnaker Vimal, K., Rekha, R., Pai Asha, K.B., Ganesh, H.R. & Krishnaprasad, M.S. (2012) Rapid slide culture: Relevance to the modern day diagnosis of tuberculosis. *Journal of Clinical Diagnostic Research*, 6(3), 378-381.

Saubolle, M.A. & Sussland, D. (2003) Nocardiosis: Review of clinical and laboratory experience. *Journal of Clinical Microbiology,* 41(10), 4497-4501.

Schlaak, C., Hoffmann, P., May, K. & Weimann, A. (2005) Desalting minimal amounts of DNA for electroporation in *E.coli*: a comparison of different physical methods. *Biotechnology Letter,* 27, 1003-1005.

Schneider, P.M., Balogh, K., Naveran, N., Bogus, M., Bender, K., Lareu, M. & Carracedo, A. (2004) Whole genome amplification-the solution for a common problem in forensic casework? *International Congress Series,* 1261, 24-26.

Schoepf, K., Prodinger, W.M., Glawischnig, W., Hofer, E., Fernandez, S.R., Hofrichter, J., Fritz, J., Kofer, J. & Schmoll, F. (2012) A two-years' survey on the prevalence of tuberculosis caused by *Mycobacterium caprae* in red deer (*Cervus elaphus*) in the Tyrol, Austria. *International Scholarly Research Network,* 2012(2012), Article ID 24513.

See, M.J., Staggs, S.E., Dubey, J.P. & Villegas, E.N. (2012) Evaluation of four RNA extraction methods for gene expression analyses of *Cryptosporidium parvum* and *Toxoplasma gondii* oocysts. *Journal of Microbiological Methods,* 89, 185-192.

Selva, E., Hofman, V., Berto, F., Musso, S., Catillo, L., Santini, J., Dellamonica, P. & Hofman, P. (2004) The value of polymerase chain reaction detection of *Mycobacterium tuberculosis* in granulomas isolated by laser capture microdissection. *Pathology,* 36(1), 77-81.

Selvakumar, N., Gomathi, M., Rehman, F. & Narayanan, P.R. (2002a) Evaluation of a two-reagent cold staining method for detection of acid-fast bacilli. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease,* 6(8), 728-731.

Selvakumar, N., Rahman, F., Rajasekaran, S., Narayanan, P.R. & Frieden, T.R. (2002b) Inefficiency of 0.3% carbol fuchsin in Ziehl-Neelsen staining for detecting acid-fast bacilli. *Journal of Clinical Microbiology,* 40(8), 3041-3043.

Senkoro, M., Mfinanga, S.G. & Mørkve, O. (2010) Smear microscopy and culture conversion rates among smear positive pulmonary tuberculosis patients by HIV status in Dar es Salaam, Tanzania. *BMC Infectious Diseases,* 10, 210.

Shah, N.S., Lan, N.T.N., Huyen, M.N.T., Laserson, K., lademarco, M.F., Binkin, N., Wells, C. & Varma, J.K. (2009) Validation of the line-probe assay for rapid detection of rifampicin-resistant *Mycobacterium tuberculosis* in Vietnam. *International Journal of Tuberculosis and Lung Disease,* 13(2), 247-252.

Shalem, O., Dahan, O., Levo, M., Martinez, M.R., Furman, I., Segal, E. & Pilpel, Y. (2008) Transient transcriptional responses to stress are generated by opposing effects of mRNA production and degradation. *Molecular Systems Biology,* 4(4), 10.1038/msb.2008.59.

Silva, M.R., Guimarães, M.D.C., Oliveira, V.M.d., Moreira, A.d.S., Costa, R.R.d., Abi-Zaid, K.C.F., Rocha, A.d.S. & Suffys, P.N. (2011) Identification of *Mycobacterium tuberculosis* complex based on amplification and sequencing of the oxyR pseudogene from stored Ziehl-Neelsen-stained sputum smears in Brazil. *Memórias do Instituto Oswaldo Cruz,* 106(1), 9-15.

Simic, D., Vleet, T.V., Euler, C., Marx, P., Bunch, R.T., Peden, W.M. & Sanderson, T. (2011) An alternative method for high throughput RNA isolation from whole blood. *Journal of Medical Genetics and Genomics,* 3(4), 77-76.

Simone, N.L., Bonner, R.F., Gillespie, J.W., Emmert-Buck, M.R. & Liotta, L.A. (1998) Laser-capture microdissection: opening the microscopic frontier to molecular analysis. *Trends in Genetics,* 14(7), 272-276.

Small, P. (2012) Why India should become a global leader in high-quality, affordable TB diagnostics. *Indian Journal of Medical Research,* 135(May), 685-689.

Soini, H. & Musser, J.M. (2001) Molecular diagnosis of mycobacteria. *Clinical Chemistry,* 47(5), 809-814.

St Amand, A.L., Frank, D.N., De Groote, M.A., Basaraba, R.J., Orme, I.M. & Pace, N.R. (2005a) Use of specific rRNA oligonucleotide probes for microscopic detection of *Mycobacterium tuberculosis* in culture and tissue specimens. *Journal of Clinical Microbiology,* 43(10), 5369-5371.

St Amand, A.L., Frank, D.N., De Groote, M.A. & Pace, N.R. (2005b) Use of specific rRNA oligonucleotide probes for microscopic detection of *Mycobacterium avium complex* organisms in tissue. *Journal of Clinical Microbiology,* 43(4), 1505-1514.

Stahlberg, A., Kubista, M. & Pfaffl, M. (2004) Comparison of reverse transcriptases in gene expression analysis. *Clinical Chemistry,* 50(9), 1678-1680.

Steege, D.A. (2000) Emerging features of mRNA decay in bacteria. *RNA,* 6, 1079-1090.

Steingart, K.R., Henry, M.N.,V., Hopewell, P.C., Ramsay, A., Cunningham, J., Urbanczik, R., Perkins, M., Aziz, M.A. & Pai, M. (2006a) Fluorescence versus conventional sputum smear microscopy for tuberculosis: A systematic review. *The Lancet Infectious Diseases,* 6, 570-581.

Steingart, K.R., Ng, V., Henry, M., Hopewell, P.C., Ramsay, A., Cunningham, J., Urbanczik, R., Perkins, M.D., Aziz, M.A. & Pai, M. (2006b) Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: A systematic review. *The Lancet Infectious Diseases,* 6(10), 664-674.

Stephan, J., Bender, J., Wolschendorf, F., Hoffmann, C., Roth, E., Mailänder, C., Engelhardt, H. & Niederweis, M. (2005) The growth rate of *Mycobacterium smegmatis* depends on sufficient porin-mediated influx of nutrients. *Molecular Microbiology,* 58(3), 714-730.

Stout, J.E., Gadkowski, L.B., Rath, S., Alspaugh, J.A., Miller, M.B. & Cox, G.M. (2011) Pedicure-associated rapidly growing mycobacterial infection: An endemic disease. *Clinical Infectious Diseases,* 53(8), 787-792.

Su, W., Feng, J., Chiu, Y., Huang, S. & Lee, Y. (2011) Role of 2-month sputum smears in predicting culture conversion in pulmonary tuberculosis. *European Respiratory Journal,* 37(2), 376-383.

Sunnotel, O., Snelling, W.J., Xiao, L., Moule, K., Moore, J.E., Millar, B.C., Dooley, J.S.G. & Lowery, C.J. (2006) Rapid and sensitive detection of single

Cryptosporidium oocysts from archived glass slides. *Journal of Clinical Microbiology,* 44(9), 3285-3291.

Suzuki, K., Takigawa, W., Tanigawa, K., Nakamura, K., Ishido, Y., Kawashima, A., Wu, H., Akama, T., Sue, M., Yoshihara, A., Mori, S. & Ishii, N. (2010) Detection of *Mycobacterium leprae* DNA from archaeological skeletal remains in Japan using whole genome amplification and polymerase chain reaction. *PloS ONE,* 5(8), e12422.

Sweeney, K.A., Dao, D.N., Goldberg, M.F., Hsu, T., Venkataswamy, M.M., Henao-Tamayo, M., Ordway, D., Sellers, R.S., Jain, P., Chen, B., Chen, M., Kim, J., Lukose, R., Chan, J., Orme, I.M., Porcelli, S.A. & Jacobs, W.R.,Jr (2011) A recombinant Mycobacterium smegmatis induces potent bactericidal immunity against *Mycobacterium tuberculosis*. *Nature Medicine,* 17(10), 1261-1268.

Taeksun, S., Woong, P.S., Su-Jeong, P., Hyang, K.J., Yu, J.Y., Oh, J.I. & Kim, Y.M. (2010) Cloning and expression analysis of the duplicated genes for carbon monoxide dehydrogenase of *Mycobacterium* sp. strain JC1 DSM 3803. *Microbiology,* 156(4), 999-1008.

Tang, Y.J., Shui, W., Myers, S., Feng, X., Bertozzi, C. & Keasling, J.D. (2009) Central metabolism in *Mycobacterium smegmatis* during the transition from O2-rich to O2-poor conditions as studied by isotopomer-assisted metabolite analysis. *Biotechnology Letters,* 31(8), 1233-1240.

Tavast, E., Salo, E., Seppala, I. & Tuuminen, T. (2009) IGRA tests perform similarly to TST but cause no adverse reactions: Pediatric experience in Finland. *BMC Research Notes,* 2(1), 9.

Tebaldi, N.D., Peters, J., Souza, R.M., Chitarra, L.G., van der Zouwen, P., Bergervoet, J. & van der Wolf, J. (2010) Detection of *Xanthomonas axonopodis pv. phaseoli* in bean seeds by flow cytometry, immunostaining and direct viable counting. *Tropical Plant Pathology,* 35(4), 213-222.

Telenti, A., Imboden, P., Marchesi, F., Schmidheini, T. & Bodmer, T. (1993) Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by

polymerase chain reaction and single-strand conformation polymorphism analysis. *Antimicrobial Agents and Chemotherapy*, 37(10), 2054-2058.

Toney, N.C., Toney, S.R. & Butler, W.R. (2010) Utility of high-performance liquid chromatography analysis of mycolic acids and partial 16S rRNA gene sequencing for routine identification of *Mycobacterium* spp. in a national reference laboratory. *Diagnostic Microbiology and Infectious Disease,* 67(2), 143-152.

Tortoli, E., Mariottini, A. & Mazzarelli, G. (2003) Evaluation of INNO-LiPA MYCOBACTERIA v2: Improved reverse hybridization multiple DNA probe assay for mycobacterial identification. *Journal of Clinical Microbiology*, 41(9), 4418-4420.

Tu, H.Z., Chen, C.S., Huang, T.S., Huang, W.K., Chen, Y.S., Liu, Y.C. & Lin, Y.E. (2007) Use of a disposable water filter for prevention of false-positive results due to nontuberculosis mycobacteria in a clinical laboratory peforming routine acid-fast staining for tuberculosis. *Applied and Environmental Microbiology,* 73(19), 6296-6298.

Uda, A., Tanabayashi, K., Osamu, F., Hotta, A., Yamamoto, Y. & Yamada, A. (2007) Comparison of whole genome amplification methods for detecting pathogenic bacterial genomic DNA using microarray. *Japan Journal of Infectious Diseases,* 60, 355-361.

Unnimaran, S., Chatterji, M. & Nagaraja, V. (2002) A hairpin near the 5' end stabilises the DNA gyrase mRNA in *Mycobacterium smegmatis.*. *Nucleic Acids Research,* 30(24), 5376-5381.

USAID 2010, *Building Partnerships to Control Tuberculosis.*, United States Agency International Development (USAID), Washington DC.

Valente, W.J., Pienaar, E., Fast, A., Fluitt, A., Whitney, S.E., Fenton, R.J., Barletta, R.G., Chacon, O. & Viljoen, H.J. (2009) A kinetic study of *in vitro* lysis of *Mycobacterium smegmatis*. *Chemical Engineering Science,* 64(9), 1944-1952.

van der Zanden, A.G.M., Te Koppele-Vije, E.M., Bhanu,N.V.,Soolingen,D.V. & Schouls, L.M. (2003) Use of DNA extracts from Ziehl-Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology,* 41(3), 1101-1108.

Vijdea, R., Stegger, M., Sosnovskaja, A., Andersen, A.B., Thomsen, V.Ø. & Bang, D. (2008) Multidrug-resistant tuberculosis: Rapid detection of resistance to rifampin and high or low levelsof isoniazid in clinical specimens and isolates. *European Journal of Clinical Microbiology & Infectious Diseases,* 27(11), 1079-1086.

Wagner, J., Short, K., Catto-Smith, A.G., Cameron, D.J.S. & Bishop,R.F.,Kirkwood,C.D. (2008) Identification and characterisation of *Pseudomonas* 16S Ribosomal DNA from ileal biopsies of children with Crohn's disease. *PLoS ONE,* 3(10), e3578.

Walker, G.T., Fraiser, M.S., Schram, J.L., Little, M.C., Nadeau, J.G. & Malinowski, D.P. (1992) Strand displacement amplification-an isothermal, *in vitro* DNA amplification technique. *Nucleic Acids Research,* 20(7), 1691-1696.

Wallis, R.S., Pai, M., Menzies, D., Doherty, T.M., Walzl, G., Perkins, M.D. & Zumla, A. (2010) Biomarkers and diagnostics for tuberculosis: progress, needs and translation into practice. *Lancet,* 375, 1920-1937.

Wang, H.X., Yue, J., Han, M., Yang, J.H., Gao, R.L., Jing, L.J., Yang, S.S. & Zhao, Y.L. (2010) Nontuberculous mycobacteria: Susceptibility pattern and prevalence rate in Shanghai from 2005 to 2008. *China Medical Journal,* 123(2), 184-187.

Watterson, S.A. & Drobniewski, F.A. (2000) Modern laboratory diagnosis of mycobacterial infections. *Journal of Clinical Pathology,* 53(10), 727-732.

Wilson, M.L. (2011) Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clinical Infectious Diseases,* 52(11), 1350-1355.

Wittliff, J.L. & Erlander, M.G. (2002) Laser capture microdissection and its applications in genomics and proteomics. *Methods in Enzymology*, 356(Laser Capture Microscope), 13.

Woods, G.L. (2001) Molecular Techniques in Mycobacterial Detection. *Archives of Pathology & Laboratory Medicine*, 125(122), 126.

Woyke, T., Tighe, D., Mavromatis, K., Clum, A., Copeland, A., Schackwitz, W., Lapidus, A., McCutcheon, J.P., McDonald, B.R., Moran, N.A., Bristow, J. & Cheng, J. (2010) One bacterial cell, one complete genome. *PLoS ONE*, 5(4), e10314.

Wu, N., Zhang, Y., Fu, J., Zhang, R., Feng, L.H.,Y., Li, X., Lu, N., Zhao, X., Pan, Y., Li, J., Zhu, B. & Wan, K. (2012) Performance assessment of a novel two-step multiple displacement amplification-PCR assay for detection of *Mycobacterium tuberculosis* complex in sputum specimens. *Journal of Clinical Microbiology*, 50(4), 1443-1445.

Xu, C., Houck, J.R., Fan, W., Wang, P., Chen, Y., Upton, M., Futran, N.D., Schwartz, S.M., Zhao, L.P., Chen, C. & Mendez, E. (2008) Simultaneous isolation of DNA and RNA from the same cell population obtained by laser capture microdissection for genome and transcriptome profiling. *Journal of Molecular Diagnostics*, 10(2), 129-134.

Zager, E. & McNerney, R. (2008) Multidrug-resistant tuberculosis. *BMC Infectious Diseases*, 8(1), 10.

Zakham, F., Akrim, M., El Mzibri, M., Benjouad, A., El Aouad, R. & Ennaji, M.M. (2012) Rapid screening and diagnosis of tuberculosis: A real challenge for the mycobacteriologist. *Cellular and Molecular Biology (Noisy-le-Grand, France)*, 58 Suppl, OL 1632-40.

Zakham, F., Belayachi, L., Ussery, D., Akrim, M., Benjouad, A., El Aouad, R. & Ennaji, M.M. (2011) Mycobacterial species as case-study of comparative genome analysis. *Cell and Molecular Biology*, 57(supp), 1462-1469.

Zellweger, J.P., Zellweger, A., Ansermet, S., de Senerclens, B. & Wrighton-Smith, P. (2005) Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than tuberculin skin test. *International Journal Tuberculosis and Lung Disease*, 9, 1242-47.

Zignol, M., Hosseini, M.S., Wright, A., Weezenbeek, C.L., Nunn, P., Watt, C.J., Williams, B.G. & Dye, C. (2006) Global incidence of multidrug-resistant tuberculosis. Journal of Infectious Diseases, 194, 479-485.

Zink, A.R. & Nerlich, A.G. (2004) Molecular strain identification of the *Mycobacterium tuberculosis* complex in archival tissue samples. *Journal Clinical Pathology*, 57, 1185-1192.