Comparison of Quantiferon-TB Assay with Conventional Methods for Diagnosis of Genitourinary Tuberculosis

Thesis for the partial Fulfillment for the M.D Degree in Microbiology and Immunology

By

MOHAMED MORSHEDY ALDESOKY ISMAEL
Assistant lecturer of Microbiology and Immunology
Faculty of Medicine (Damietta) – Al -Azhar University

SUPERVISED BY

Dr. AHMED OSMAN EL-KAFRAWI
Professor and head of Microbiology and Immunology
Faculty of Medicine – Al-Azhar University

Dr. MOSTAFA YOSEF ALI EL-MISHAD
Professor of Microbiology and Immunology
Faculty of Medicine – Al-Azhar University

Dr. SOMIA ABD EL-LATIF ESSA
Professor of Microbiology and Immunology
Faculty of Medicine – Cairo University

Dr. HAMDI ABDEL HAMED ABD ELKADER
Professor of Urology
Faculty of Medicine – Al-Azhar University

Dr. MOHAMED MOHAMED AHMED SALEH
Assistant Professor of Microbiology and Immunology
Faculty of Medicine (Damietta) – Al-Azhar University

Faculty of Medicine
Al-Azhar University
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List of abbreviations

Abbreviation	Full meaning
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
ADA	Adenosine Deaminase
APC	Antigen Presenting Cells
BCG	Bacillus Calmette Guirine
CDC	Centers for Disease Control and Prevention
CXR	Chest X-Ray
CV	Coefficient of Variation
CBC	Complete Blood Count
CT	Computerized Tomography
CFP10	Culture Filtrate Protein 10
DTH	Delayed Type Hypersensitivity
DOTS	Directly Observed Treatment Short course
ESAT6	Early Secreted Antigenic Target
ELISA	Enzyme Linked Immunosorbant Assay
ELISPOT	Enzyme-linked Immunospot
ESR	Erythrocyte Sedimentation Rate
EPTB	Extrapulmonary Tuberculosis
XDR-TB	extremely drug-resistant tuberculosis
FGTB	Female Genital Tuberculosis
FNAC	Fine Needle Aspiration Cytology
FDA	Food and Drug Administration
GU	Genitourinary
GUTB	Genitourinary tuberculosis
GD	Green Diluent
GI	Growth Index
HIV	Human Immunodeficiency Virus
HLA	Human Leucocytic Antigen
ICT	Immunochromatographic test kit
IT	In Tube
IVU	Interavenous Urography
IGRA	Interferon Gamma Release Assays
INF-γ	Interferon-γ
IL-2	Interleukin-2

IVP	Intra Venous Pylography
KAN	Kanamycin
LTBI	Latent Tuberculosis Infection
LAMP	Loop-mediated isothermal Amplification
LJM	Lowenstein-Jensen Medium
MRI	Magnetic Resonance Image
MHC	Major Histocombitability
MODS	Microscopic Observation Drug Susceptibility
MDR	Multidrug Resistant
MOTT	Mycobacteria Other Than Tuberculosis
MAC	Mycobacterium avium complex
M. bovis	*Mycobacterium bovis*
MGIT	Mycobacterium Growth Indicator Tube
MTB	*Mycobacterium Tuberculosis*
M. tuberculosis	*Mycobacterium Tuberculosis*
MTD	*Mycobacterium tuberculosis* Direct Test
NALC	N-acetyl-L-cysteine
NTP	National Tuberculosis Control Program
NPV	Negative Predictive Value
NAA	Nucleic Acid Amplification
OD	Optical Density
OFL	Ofloxacin
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
PPD	Purified Protein Derivative
QFT-G	QuantiFERON-TB Gold
RD1	Region of Difference 1
RFLP	Restriction Fragment Length Polymorphism
rRNA	ribosomal RNA
SLDs	Second line drugs
NaOH	Sodium hydroxide
TST	Tuberculin Skin Test
TB	Tuberculosis
TNF-α	Tumor Necrosis Factor Alpha
UV	Ultraviolet

USA	United States of America
UO	Ureteric Orifice
UTP X ray	Urinary Tract Plain X ray
VUR	Vesico Ureteric Reflux
WBCs	White blood cells count
WHO	World Health Organization
Z-N	Ziehl-Neelsen

Introduction

Pulmonary tuberculosis was known since the time of Hippocrates as “phthisis”, which is derived from the Greek meaning consumption or wasting away. Later, Aristotle and Galen recognized that Tuberculosis was transmissible ***(Dormandy, 1999).*** The Tuberculosis epidemic in Europe, later known as the “Great White Plague”, started at the beginning of the 17th century. In 1689, the term “consumption” was used to denote Tuberculosis (TB) ***(Ducati et al., 2006).*** Tuberculosis was called consumption, because it seemed to consume people from within, with a bloody cough, fever, pallor, and long relentless wasting ***(Rudy’s List of Archaic Medical Terms, 2006).***

Pulmonary tuberculosis in an Egyptian mummy was diagnosed & confirmed by Polymerase Chain Reaction (PCR) & this was probably the first confirmed case of pulmonary tuberculosis using PCR in an ancient Egyptian mummy ***(Nerlich et al., 1997)***.

In ***1882, Robert Koch*** discovered *Tubercle bacillus*, and in ***1895, Wilhelm Röntgen*** discovered X-rays. These scientific triumphs were quickly applied to clinical medicine, so that around 1905, doctors could make a precise diagnosis of consumption. The steady fall in the incidence of TB was confounded by a steep rise during and after the two world wars ***(Gillespie, 2006).***

Since the mid-1980s, however, this decreasing trend slowed down and even reversed in some countries, such as the United States of America (USA). The resurgence of the disease was attributed to the epidemic of Human Immunodeficiency Virus (HIV) infection, diminished public health efforts to control TB, rising poverty, homelessness, overcrowded conditions, and immigration from countries with a high prevalence of TB ***(Lienhard, 2001).***

Tuberculosis has recently reemerged as a major health concern. Each year, approximately 2 million persons worldwide die of tuberculosis and 9 million become infected ***(Nancy et al., 2009).***

From the most common forms of extra pulmonary TB is genitourinary disease, accounting for 27% (range, 14 to 41%) worldwide ***(Prasenjit et al., 2008)***. Genitourinary tuberculosis (GUTB) is the second most common form of extrapulmonary tuberculosis, with more than 90% of cases occurring in developing countries ***(Aula et al., 2011).***

Genitourinary tuberculosis represents a challenge in diagnosis and treatment due to variations in clinical and radiological signs, patient history and difficulty in the isolation of the bacilli ***(Aslan et al., 2007)***.

The difficulty in diagnosis is due to the lack of efficient and sensitive diagnostic tools as well as its variable anatomical location. Symptoms are insidious and non-specific. Symptoms typical of active tuberculosis such as fever, weight loss, cough and haemoptysis are uncommon in patients with genitourinary tuberculosis. The most common symptoms, if present are frequency (60%) which is usually intermittent, followed by dysuria (34%) and haematuria (28%). The disease often presents with symptoms of bladder inflammation in (21%) of patients, haemospermia with or without necrospermia in males and tubal obstructions in females. Complication of genitourinary tuberculosis includes renal calcification, hypertension, stricture, non-specific bacterial infection, impaired renal function and sterility. Early diagnosis of cases by simple laboratory technique may give the best chance of treatment before major complications are established. There are very few diagnostic tools from which to choose. Microscopy is the most rapid diagnostic tool, which in ideal settings can produce same day results, but it is very insensitive. Culture systems are sensitive, but often take up to four weeks to obtain conclusive results even with enhanced culture systems and many culture with false

negative results due to intermittent passage of the organism. More sensitive and rapid TB diagnosis is not yet available ***(Edford et al., 2009)***.

It has been difficult to develop an Enzyme Linked Immunosorbant Assay (ELISA) utilizing a suitable antigen because *Mycobacterium tuberculosis* (MTB) shares a large number of antigenic proteins with other microorganisms that may or may not be pathogenic. The PCR results must be corrected for the presence of inhibitors as well as for DNA contamination ***(Sanjay et al., 2003).***

Most of the published studies compared the performance of in vitro Interferon-γ (INF-γ) release assays with Tuberculin Skin Test (TST) for detection of Latent *Tubercle Bacilli* Infection (LTBI). Judging from currently accumulated research experience, in vitro INF-γ release assays are likely to be promising alternatives to the TST in the diagnosis of LTBI. However, their performance in diagnosis of active disease is still under study ***(Chee et al., 2008).***

The Centers for Disease Control and Prevention (CDC) has published new TB detection guidelines, advising that QuantiFERON(R)-TB Gold, a simple, one-step blood test that can be used as a rapid diagnosis of tuberculosis infections, for early detection and control of disease***(CDC, 2006).***

Enzyme-linked Immunospot (ELISPOT) and ELISA techniques have been developed to rapidly detect IFN-γ production by M TB-specific Peripheral Blood Mononuclear Cells (PBMCs) for the diagnosis of MTB infection. ***(Pai et al., 2007).***

QuantiFERON-TB Gold include 6 kDa Early Secreted Antigenic Target (ESAT6) and Culture Filtrate Protein 10 (CFP10) which are both encoded by the region of difference 1 (RD1) which is present in *Mycobacterium tuberculosis (M. tuberculosis)* and *Mycobacterium bovis (M. bovis)*, but absent from *M. bovis* Bacillus Calmette Guirine (BCG) and most

environmental mycobacteria. ESAT6 is an immunodominant T cell-stimulatory antigen and is recognized by specific IFN-γ secreting T cells present in greater numbers in patients with active disease as compared with those who are un-infected The sensitivity of ESAT6 and CFP10 induced mycobacterium-specific T cell responses is greatest in a BCG unvaccinated population in a non-endemic region, and most studies have been performed in areas of low tuberculosis transmission with less data available from high transmission TB endemic regions ***(Zahra et al., 2009).***

The whole-blood interferon-gamma enzyme-linked immuno-sorbent assay (QuantiFERON-TB Gold [QFT-G]; has been studied mainly for diagnosing active pulmonary tuberculosis or latent TB ***(Kyoung et al., 2009).***