COMPARISON BETWEEN CONVENTIONAL METHODS FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS AND PCR USING TWO DIFFERENT PROTOCOLS FOR DNA EXTRACTION

A thesis submitted for partial fulfillment of MD degree in basic medical Science (Microbiology and Immunology)

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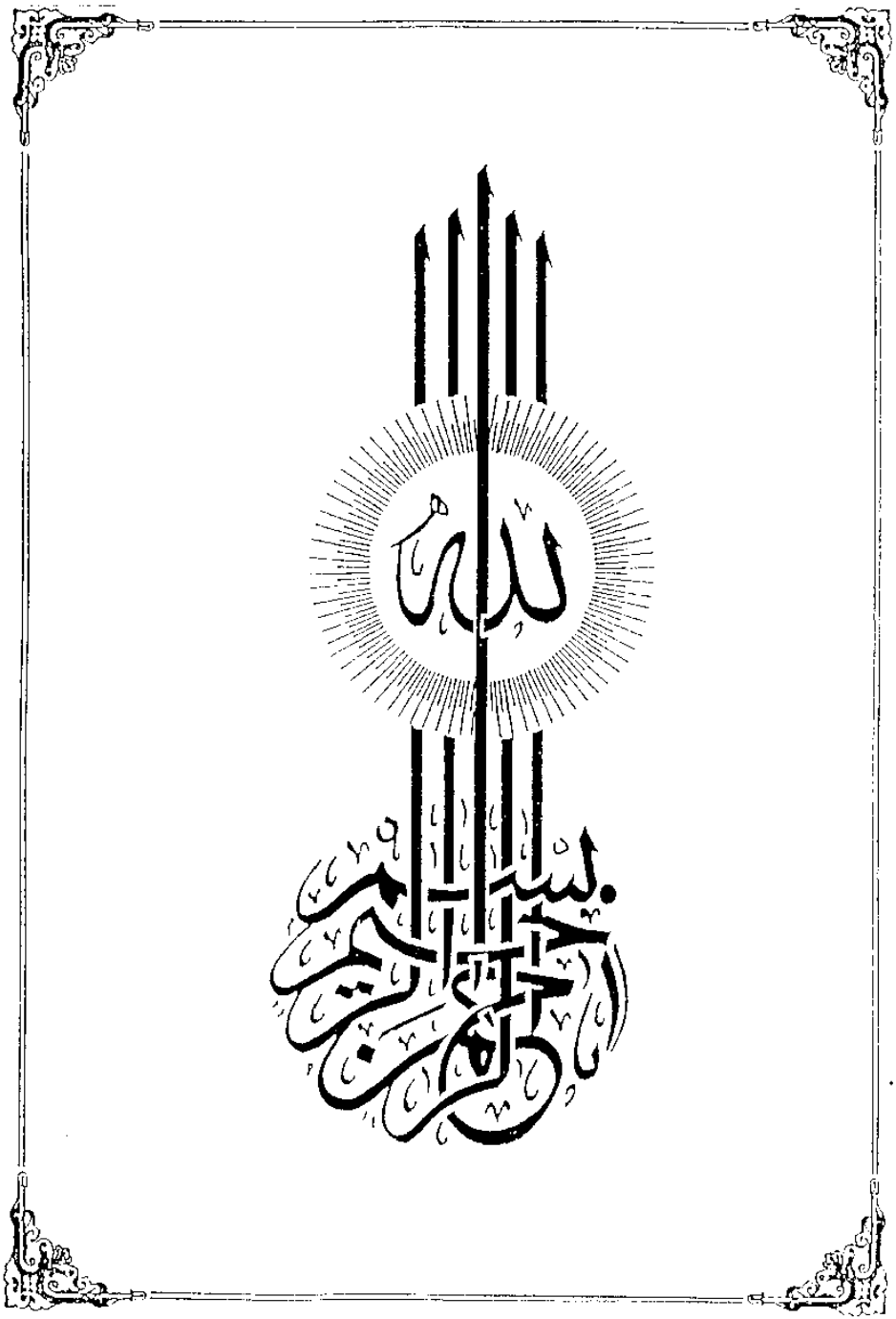

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Dedication

To my parents and my brothers, who saved no effort ,money or support to make my life more comfortable and productive , even on the expense of their own happiness.

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List of Abbreviations

AFB	Acid fast bacilli
APC	Antigen presenting cell
ATC	American thoracic society
BAL	Bronchoalveolar lavage
BCG	Bacille Calmette-Guerrin
CDC	Center for disease control and prevention
CFUs	Colony forming units
CMI	Cell mediated Immunity
CSF	Cerebrospinal fluid
DHR	Delayed hypersensitivity reaction
DR	Direct repeat
DS	Double stranded
DTH	Delayed hypersensitivity
EIA	Enzyme immuoassay
ELISA	Enzyme linked immunosorbent assay
GC-MS	Gas chromatography-mass spectrum
GI	Growth index
GM-CSF	Granulocyte monocyte-colony stimulating factor
HPLC	High performance liquid chromatography
I.C	Intracutaneously
IC	Immune complex
IFN	Interferon
IL	Interleukin
INH	Isoniazid
IUATLD	International union against tuberculosis and lung diseases
KD	Kilo dalton
LAM	Lipoarabinomannan
LCR	Ligase chain reaction
LJ	Lownstein Jensen
M.TB	Mycobacterium tuberculosis

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INTRODUCTION

Introduction

The recent resurgence in tuberculosis cases poses a serious public health problem because of its increased incidence and emergence of multi-drug resistant strains. The WHO reported eight million new cases of TB with 2.9 million deaths in 1990. HIV infection makes the tuberculosis problem more serious in both developed and developing countries due to i - creased incidence of compromised hosts and as manifestations in AIDS patients, therefore, a correct diagnosis of mycobact - rial diseases is important and early detection of the organism in clinical samples becomes more and more important in the control of tuberculosis ***(Perriens et al 1995).***

The currently accepted gold standard laboratory method for detecting and identifying Mycobacterium tuberculosis (M.TB) is a combination of acid fast bacillus (AFB) smear for initial screening and culture for bacterial isolation and identification. Although these methods are continuously refined and improved, they still have severe limitations ***(Schluger et al 1995).*** Microscopic examination of AFB smears can yield a result within 24 hours, but it is neither specific nor very sensitive, requiring more than 10^3 to 10^4 organisms per ml of sputum to be positive. However, because of their rapidity, they are used to manage patients and to make public health decisions ***(Estelle et al 1995).*** M.TB can also be detected and identified directly from sputum specimens by analyzing the Mycobacterium mycolic acid by HPLC. However, the sensitivity of this method is low and only possible in smear positive specimens ***(Vincent et al 1995).***

Bacterial culture is superior to AFB smear and mycolic acid analysis, both in terms of sensitivity and specificity. However, M.TB are fastidious organisms with very strict growth requirements. As a result, culture based methods for diagnosis is slow and laborious. Clinical laboratories hold cultures for six to eight weeks to achieve the maximum sensitivity. Some AFB

REVIEW

Mycobacterium

Taxonomy:

The genus Mycobacterium is the only genus in the family Mycobacteriaceae. The high G+C content of the DNA of mycobacteria (62% to 70%) is similar to that of the other mycolic acid-producing bacteria , Nocardia (60% to 69%), Rhodococcus (59% to 69%) and Corynebacterium (51% to 59%) spp. This similarity may support the consolidation of these genera into a single family ***(Wayne and Kubica, 1986).***

The generic name Mycobacterium (fungus bacterium) was proposed by ***Lehmann and Neumann (1869)*** in reference to the mould like pellicle formed by M. tuberculosis on liquid media ***(Watt et al,1996).***

Description of the genus:

The mycobacteria are slightly curved or straight bacilli , 0.2-0.6 *um* wide and 1 to 10 *um* long, but they also may appear coccoid or filamentous (long , slender and even bracnching). They are frequently bent and may contain heavily stained areas called beads or alternating stained and clear areas that make them appear banded . From pure cultures individual rods of M. tuberculosis (M.TB) may be aggregated side by side and end to end to form "cords". This is often the case in smears made from the turbid fluid at the bottom of a lowenstein-Jensen slant on which M.TB is growing (fig I-1) ***(Kent and Kubica ,1985).*** Mycobacteria have cell walls with a high lipid content that includes waxes having characteristic mycolic acid with long, branched chains. The high lipid content of the cell wall e - cludes the usual aniline dyes.

Mycobacteria are not easily stained by the gram method. Special staining procedures are used to promote the uptake of dye and once stained ,mycobacteria are not easily decolorized even with acid-alcohol. This resistance to decolorization by acid alcohol is termed acid fastness. Acid fastness may be

ammonia or aminoacids as nitrogen sources and glycerol as a carbon source in the presence of mineral salts. Some species require medium supplements such as hemin, mycobactins, or other iron transport compounds. M. Leprae has not been cu - tured outside of living cells.

The species of Mycobacteria:

I. M. tuberculosis complex:

M. tuberculosis complex includes the species M. tuberculosis, M. bovis, M. microti and M. africanum. ***Robert Koch*** was the first to establish the causal relationship between the tubercle bacillus and the disease tuberculosis (TB). The organism was named M. tuberculosis in ***1886***, presumably because it resembled a fungus in its slow growth and colony morphology ***(Notle and Metchock, 1995)***.

M. bovis was the name given to the bovine tubercle bacilli in 1896. M. bovis causes tuberculosis in cattle ,humans and other primates, carnivoras, including dogs and cats ,swine ,parrots and some birds. The disease produced in humans is virtually indistinguishable from that caused by M. tuberculosis and is treated similarly.

The bacillus of Calmette-Guerin (BCG) which is used as a vaccine against tuberculosis in many parts of the world, conforms the properties described for M. bovis except that its pathogenicity is more attenuated.

M. africanum may represent an intermediate form between M. tuberculosis and M. bovis, and retention of M. africanum as a distinct species is probably not justified . M. africanum is a cause of human TB in tropical Africa . M. microti also occupies a position along the phenotypic continuum between M. tuberculosis and M. bovis, and it causes naturally acquired generalized TB in the vole and produces local lesions in guinea pigs, rabbits and calves.

differences in the types of disease caused by or the antibiotic resistance patterns of the different components of this complex. DNA probes are available that distinguish between M. avium and M. intracellulare. In the MAC kit currently available for diagnostic use, however, these 2 probes plus an "X" cluster probe are combined together, so that with this kit an isolate can only be identified as belonging to the MAC as a whole. M. scrofulaceum is sometimes treated as if it were closely related to the MAC, and sometimes the designation M. avium-intracellulare-scrofulaceum(MAIS) may be used to refer to isolates of uncertain position within this group of organisms. In fact, M. scrofulaceum doesn't appear to be phylogenetically close to any of the members of MAC ***(Wayne and Sramek, 1992).*** The term MAIS intermediate which doesn't refer to any presently recognized taxonomically valid species, should only be used to refer to organisms possessing certain specific biochemical features as described by ***Hawkins, 1977.***

III. <u>M. kansasii:</u>

This is the name currently given to the photochromogenic (yellow bacillus) originally described in 1953 ***(Nolte and Metchock, 1995).*** Studies of the base sequences of the 16S rRNA and of catalase serology suggest that M. kansasii is phylogenetically very closely related to a non pathogenic, slowly growing non pigmented species , M. gastri ***(Rogall et al, 1990)***. However, the distinction between the two at the species or subspecies level should be maintained because of the clinical significance of most isolates of M. kansasii. The taxonomy of mycobacteria continues to evolve as molecular method of distinguishing species become more sophisticated ***(Nolte and Metchock, 1995).***

M. kansasii is a well recognized human pathogen that is now relatively less frequently isolated as a result of the rising incidence of MAC isolates . DNA probe is commercially available for the identification of M. kansasii, although in one study