About IKP Centre for Technologies in Public Health

IKP Centre for Technologies in Public Health (ICTPH) is a research centre that aims to improve the health of poor populations by focusing on designing, developing and delivering innovative solutions in healthcare concerning India and the developing world through an inclusive process that scientifically integrates knowledge of factors influencing health and diseases in India, regular evaluation and impact assessment of existing health systems and integration of appropriate technology for optimal health care delivery. ICTPH aims to learn, discover and apply relevant innovative solutions for health care leading to improved health for the people of India and other developing countries and to integrate technological advances with delivery of affordable, accountable and accessible health care. ICTPH has prioritized the diseases of its interest as malaria, tuberculosis, reproductive health, diarrhoeal diseases and diabetes.

IKP Centre for Technologies in Public Health is structured as an autonomous centre within ICICI Knowledge Park (IKP), a not-for-profit Research Park in Hyderabad, India focusing on Life Sciences.

City Office:
ICICI Knowledge Park
ICICI Bank Tower
1-11-256, Level IV
St. No. 1, Begumpet
Hyderabad - 500 016
India

Site Office:
ICICI Knowledge Park
Genome Valley
Turkapally
Shameerpet, RR Dist.
Hyderabad – 5000 078
India

Tel: +91 40 2348 0111
e-mail: info@ictph.org.in
Fax: +91 40 2348 0007
www.ictph.org.in

Foreword

The IKP Centre for Technologies in Public Health (ICTPH) aims to identify the most important evidence gaps in the current knowledge that India and developing countries have about health technologies pertinent to diseases of interest. ICTPH has prioritized the diseases of its interest as malaria, tuberculosis, reproductive health, diarrhoeal diseases and diabetes. Pertinent to Tuberculosis, ICTPH is interested in the current status of preventive, curative and therapeutic interventions with a focus on determining gaps in knowledge as well as potential areas for improvement. This report will be utilized by ICTPH to explore potential product development and/or development of product diffusion and scale up strategies. We welcome comments and suggestions from our readers.

Reports on strategies for malaria, reproductive health, diarrhoeal diseases and diabetes are also available as a part of our Working Paper Series.

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Deepanwita Chattopadhyay

CEO

ICICI Knowledge Park

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Executive Summary

Early and sustained intervention is the key to effective management of Tuberculosis. Early intervention is dependent on an early and accurate diagnosis of the disease. A satisfactory diagnosis in terms of accuracy and speed is difficult with culture of the organism considered as the gold standard. Culture is the most sensitive of currently available tests (sensitivity rates of up to 98% have been reported), and also permits identification and drug sensitivity tests to be made. However, it may require up to 6–8 weeks for the isolation of *M. TB* from a clinical specimen and in 10–20% of cases the bacillus is not successfully cultured. Clinical diagnosis, the most widely used approach, is unreliable because of the increasing prevalence of drug resistant tuberculosis. Microscopic diagnosis including culture, the established method for laboratory confirmation of tuberculosis, presents technical and personnel requirements that often cannot be met, particularly in facilities at the periphery of the health care system. The delays in the provision of the culture results to the clinician often result in treatment provided without the benefit of the knowledge regarding susceptibility or resistance of the organism to a particular drug.

Rapid Diagnostic Tests (RDT) in combination with current methods of diagnosis may improve early detection of tuberculosis. The ability of the tests to detect the resistant strains is of importance. The RDTs should have detection capabilities that are in general comparable to those generally achieved by microscopy in the health services. The emergence of multidrug and extreme multidrug resistance is a major incentive for the development and deployment of RDTs to obtain reliable laboratory confirmation of the disease. RDTs can be performed by local health workers or community volunteers to diagnose tuberculosis leading to early treatment. Where multidrug resistance occurs, the cost of the recommended anti-tuberculosis drugs is higher, thus justifying the use of RDTs that can detect drug resistant strains early. The need to detect multidrug resistance early is compounded by the increased case fatality among persons with drug resistant tuberculosis.

Patient failure to take the prescribed medications at the required intervals results in significant morbidity and mortality. The need for research into an oral anti-TB drug delivery system is thus warranted as the efficacy of the current regimen may be improved if the delivery rate, biodegradation, and site-specific targeting can be predicted, monitored, and controlled.

From a financial and a global health care perspective, finding new ways to administer the anti-TB drugs in oral form and delivering the multiple doses, long-term therapy in inexpensive, potent, forms with improved bioavailability is needed. The provision of an administration method, embodied by a dosage form that addresses FDC bioavailability concerns, that will allow patients to safely treat themselves and enhance their compliance with the anti-TB regimen is a significant health care development, particularly in developing countries where access to doctors, clean syringes, sterile needles, and sophisticated treatments are few and far between.

More than 200 vaccine candidates have been proposed as the result of work over recent years in experimental laboratory models, and some are in different phases of clinical testing. The transition from laboratory to clinical trials has a wide range of strategic and technical implications. In particular, facilities and funding need to be provided for the production of any successful vaccine appropriate for clinical use.

After the Madrid Conference in March 1995 ‘‘Definition of a coordinated strategy towards a new TB vaccine’’ organized by the WHO and IUATLD, a joint effort was established involving diverse governmental organizations in Europe (FP5 and FP6 Framework Programmes) and USA by National Institutes of Health and recently the Aeras Global TB Vaccine Foundation. For the first time, after 80 yrs of widespread use of BCG, evaluations of new candidates in humans are available, including recombinant vaccine virus. The development of a new vaccine conferring better protection than BCG, and able to replace it, nevertheless remains a challenge for the scientific community. Subunit vaccines have potential advantages over live mycobacterial vaccines in terms of safety and quality control of the manufactured vaccine and are good candidates to improve the effect of BCG. However, in order to confer the complex immunity required to protect against tuberculosis, it is possible that more than single antigens will be necessary. Progress to date with live attenuated Mycobacterium tuberculosis vaccines indicates that it is possible to design strains that are highly attenuated, even in immune-deficient animals. These classical vaccine candidates have to mimic natural infection as closely as possible without causing disease.

Mycobacterium tuberculosis mutant vaccine candidates have to induce long term cellular immune responses, essential for effective protection against tuberculosis.

New live vaccines should be stored lyophilized and current technology allows monitoring of any possible variations of genomic composition by comparative hybridization experiments using DNA microarrays.

DOTS (Directly Observed Therapy Short-course) programmes treated more than two million smear positive patients in a 2004 cohort, and achieved a global success rate just below the 85% target. The target was met in the South-East Asia and Western Pacific regions, and in eight High Burden Countries (HBCs). However, the overall treatment success, coupled with the 54% case detection rate in 2004, means that less than half (46%) of all new smear-positive patients were known to have been successfully treated in that cohort. Randomized controlled trials provide no assurance that the routine use of DOT in low-income and middle-income countries improves cure or treatment completion in people with tuberculosis. There is also no rigorous evidence to support the use of DOT for prophylaxis in people with latent tuberculosis. DOT is a controversial and expensive intervention, and there appears to be no sound reason to advocate its routine use until we better understand the situations in which it may be beneficial. In the meantime, it could reasonably be argued that resources should be invested in interventions that have been shown to be effective for improving adherence, such as providing patient motivation and support, incentives, and defaulter action.

There are several potential areas of interest for the ICICI Centre for Technologies in Public Health (ICTPH) in tuberculosis. The priorities are the ability to diagnose tuberculosis appropriately early and the improved ability to diagnose drug resistant tuberculosis at an early stage. These include development and deployment of RDTs including better culture media that provide rapid results. On the public health research front, there is an urgent need to explore the patient, organizational and societal impact besides cost effectiveness and cost benefits of these newer developments. The role of supplemental care for tuberculosis like nutrition although important may not at this stage be a priority for ICTPH.

A. Tuberculosis as a Public Health Priority

A.1 The Global TB Scenario

There were an estimated 8.8 million new TB cases in 2005, 7.4 million in Asia and sub-Saharan Africa. A total of 1.6 million people died of TB, including 195 000 patients infected with HIV. TB prevalence and death rates have probably been falling globally for several years. In 2005, the TB incidence rate was stable or in decline in all six WHO regions, and had reached a peak worldwide. However, the total number of new TB cases was still rising slowly, because the case-load continued to grow in the African, Eastern Mediterranean and South-East Asia regions[1]

More than 90 million TB patients were reported to WHO between 1980 and 2005; 26.5 million patients were notified by DOTS programmes between 1995 and 2005, and 10.8 million new smear-positive cases were registered for treatment by DOTS programmes between 1994 and 2004. DOTS, which underpins the Stop TB Strategy, was being applied in 187 countries in 2005; 89% of the world's population lived in areas where DOTS had been implemented by public health services. A total of 199 countries/areas reported 5 million episodes of TB in 2005 (new patients and relapses); 2.3 million new pulmonary smear-positive patients were reported by DOTS programmes in 2005, and 2.1 million were registered for treatment in 2004. WHO's 2005 targets for DOTS programmes of 70% case detection and 85% cure were narrowly missed globally: case detection was 60% (95%CL 52–69%); treatment success was 84%. However, both targets were achieved in the Western Pacific Region, and treatment success exceeded 85% in the South-East Asia Region.

Skilled and highly-motivated staff is central to any public health programme, and yet the plans for human resource development made by national TB control programmes (NTPs) in 2005–2006 were highly variable in quality. In particular, 7 of the 22 high-burden countries (HBCs), including 5 African countries, had plans that were limited in scope or under development. Prompt diagnosis and effective treatment require fully functioning laboratories and reliable drug supplies.

1. Global tuberculosis control: surveillance, planning, financing. *WHO report* **2007**. Geneva, World Health organization (WHO/HTM/TB/2007.376)

Despite some improvements, NTPs in all WHO regions reported drug stock-outs, too few laboratories, weak quality control, and limited facilities to carry out culture and drug susceptibility testing. Many NTPs asked for further technical assistance from external agencies.

Nearly 5 million TB patients were notified under DOTS in 2005, and the total number diagnosed and treated in 2006 is expected to be roughly in line with the Global Plan to Stop TB (2006–2015). However, smear-positive case detection rates by DOTS programmes varied among WHO regions in 2005, from 35% (Europe) to 76% (Western Pacific), and these variations are likely to persist into 2006. The numbers of HIV-positive and multidrug-resistant TB (MDR-TB) patients diagnosed and treated in 2005, although increasing, were far lower than proposed in the Global Plan for 2006.

HIV testing for TB patients is increasing quickly in the African Region, but little effort has yet been made to screen HIV-infected people for TB, though this is a relatively efficient method of case-finding. Facilities to diagnose and treat MDR-TB, including extensively drug-resistant TB (XDR-TB), are not yet widely available; the scale of the XDR-TB problem globally is not yet known.

The Stop TB Strategy is a mechanism for building links between NTPs, health-care providers and communities. The connections being made through community based TB care, public–private mix DOTS and the Practical Approach to Lung Health have been shown, on a small to medium scale, to improve access to diagnosis and treatment. However, no country has yet succeeded in making all of these activities fully operational at national scale. Few NTPs have an overview of TB research in their countries, and few have the skilled staff and funding needed to carry out essential operational research.

A.2 Tuberculosis in India

The World Tuberculosis Report, 2007 states that 1,156, 248 cases of tuberculosis (new and relapse) were reported in India in 2005. 1,146,599 of these cases were reported under DOTS. There were 508,890 *smear positive* cases including 506,852 covered under DOTS; 399,066 *smear negative* cases including 392,390 covered under DOTS, and 171,838 cases of *extra-pulmonary tuberculosis* including 170,948 covered under DOTS.

There were 148,580 retreatment cases of tuberculosis (excluding relapses) including 148,495 covered under DOTS. The proportion of new pulmonary smear positive cases was 56%.

The incidence of TB in India (World Tuberculosis Report, 2007) is reported as 168 new cases per 100,000 population each year in 2005; approximately 1,852,000 new cases per year. 827,000 of these are new smear positive cases (75 per 100,000 population per year). The prevalence of TB in India (World Tuberculosis Report, 2007) is 3,299,000 persons in 2005; a prevalence of 299 per 100,000 persons. An estimated 322,000 persons died in India from TB in 2005 (World Tuberculosis Report, 2007); approximately 29 per 100,000 population. The prevalence of HIV in incident TB cases was 5.2%.

The population coverage of DOTS has increased from 1.5% in 1995 to 91.0% in 2005. The case detection rate of new smear positive cases under DOTS increased from 0.3% in 1995 to 61% in 2005. The corresponding figures for the entire country were 38% and 62% respectively. The treatment outcomes for new smear positive cased under DOTS in a 2004 cohort were 84% cured; 4.4% died; 2.4% failed, and 6.6% defaulted. The treatment success rate was 86%. The proportion of estimated cases (as opposed to notified or registered cases) successfully treated under DOTS is 49%. The success rate for DOTS among retreatment smear positive cases was 73%.

India has a National Reference Laboratory for TB. In 2005, there were 11,813 labs (1.1 per 100,000 population) that were capable of examining smears; 0.02 labs per 5 million population for culture as opposed to the recommended 1 lab per 5 million population; and 0.05 labs per 10 million population that could do drug susceptibility testing as opposed to the recommended 1 lab per 10 million population. All labs were included in external quality assurance (EQA) for sputum smear microscopy.

B. Prevention of Tuberculosis – The Vaccine Approach

The lung is the portal of entry of M. tuberculosis in most human infections and provides a suitable environment for this slowly replicating pathogen. The infection is established in alveolar macrophages of the distal alveoli before it is recognized by the adaptive immune response 5–6 weeks later. CD4+ and CD8+ T cells are recruited through the lung, inducing protective immunity. Both CD4+ and CD8+ T cells are essential for protective immunity against M. tuberculosis.

Resistance to M. tuberculosis involves the activation of mycobacterial-specific CD4+ and CD8+ T cells by dendritic cells (DC), which migrate from the site of the infection in the alveoli to the draining lymph nodes. The development of interferon (IFN)-γ-secreting CD4 T cells is dependent on the secretion of interleukin (IL)-12 by infected DC. Subjects deficient in receptors for IFN-γ and IL-12 are extremely susceptible to mycobacterial infections, confirming the absolute requirement for T-helper cell type 1 (Th1)-like T cells for host immunity.[2] The nature of an effective immune response to TB is incompletely understood, but the most effective vaccination strategies in animal models are those that stimulate T-cell responses, both CD4 and CD8, to produce Th1-associated cytokines. Therefore, formulations that induce the production of enduring Th1 responses are desirable, and doubtless an essential element of a successful vaccine. Several adjuvant or live vaccines capable of inducing potent T-cell responses have been developed and some have entered clinical testing.

There are a number of substantial underlying problems to be faced in developing vaccines with enhanced protective efficacy against TB. In contrast to a classical vaccine-preventable disease such as smallpox, recovery from infection with M. tuberculosis is not associated with immunity against re-infection after clearance of the original infection with antibiotics. Studies of the molecular epidemiology of TB indicate that re-infection with new strains of TB is more frequent than previously believed.[3]

2. Flynn JL, Chan J. Immunology of tuberculosis. *Annu Rev Immunol* **2001**; *19*: 93 – 129.

3. Caminero JA, Pena MJ, Campos-Herrero MI, et al. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. *Am J Respir Crit Care Med* **2001**; *163*: 717–720.

Therefore, vaccines need to be more effective than infection with M. tuberculosis itself. One-third of the population worldwide is estimated to be infected with M. tuberculosis, and therefore any new TB vaccine should be suitable for use in subjects pre-exposure, to prevent infection, but also post-exposure, to prevent the development of disease or as an immuno-therapeutic agent to act with antimicrobials to increase the rate of clearance of M. tuberculosis. An additional challenge is that as a large percentage of the human population has already been immunized with BCG, and so any new generation vaccines against TB must also be able to protect the population that has already been vaccinated with BCG.

New vaccines must also be safe enough to be used in HIV-infected and other immune-compromised individuals.[4]

B.1 Question of Interest

What are the vaccine strategies for Tuberculosis?

Methodology

A systematic review of the scientific literature focusing on the vaccines for tuberculosis was performed. The search covered The Cochrane Library, PUBMED, MEDLINE and EMBASE as the central databases and also covered the WHO database of publications. The search was performed using one or a combination of the following key words: tuberculosis, vaccines, prevention.

Studies on adults or children with any form of tuberculosis were considered to be eligible for inclusion. No restriction was placed on study settings, and included studies from any country. Randomized controlled trials were included for the review as randomized controlled trials are considered the highest level of evidence.

Only articles in the English language were included for review. The initial review of articles was followed by a review of the cross referenced articles. The related article link in PUBMED was used sequentially to identify other similar articles of interest

B.2 The Bacillus Calmette-Guerin (BCG) Vaccine

4. Vuola JM, Ristola MA, Cole B, et al. Immunogenicity of an inactivated mycobacterial vaccine for the prevention of HIV-associated tuberculosis: a randomized, controlled trial. *AIDS* **2003**; *17*: 2351–2355.

BCG is an inexpensive vaccine that has been given to 2.5 billion people since 1948. The BCG is a live vaccine that protects against severe childhood forms of disease, including miliary and extra pulmonary TB, and the often fatal TB meningitis. BCG vaccination is currently compulsory in ≥64 countries and administered in >167 countries.[5,6]

The World Health Organization (WHO) recommends BCG vaccination in areas of high TB prevalence and incidence.

The BCG vaccine has a long-established safety profile and elicits both humoral and cell-mediated immune responses. The vaccine can be given at birth or any time thereafter. A 60-yr follow-up study in American Indians and Alaska natives has shown that the efficacy of BCG vaccine persists for 50–60 yrs, suggesting that a single dose of BCG vaccine can give life-long protection.[7] The efficacy of BCG vaccines against pulmonary TB varies between populations, showing no protection in Malawi but 50–80% protection in the UK.[8] Natural exposure to environmental mycobacteria may exert an important influence on the immune response that may mask or inhibit the effect of BCG vaccination in tropical countries.[9]

An association of TB with AIDS may affect the level of protection. The risk of disseminated BCG among adult AIDS patients with childhood BCG immunization is very low, and, in addition, childhood BCG immunization is associated with protection of adults with advanced AIDS against bacteremia with M. tuberculosis.

Studies in Zambia have shown that bacteremia due to BCG or M. tuberculosis is rare among children with BCG immunization (even recent) and symptomatic HIV infection.[10]

5. World Health Organization. W. H. O. statement on BCG revaccination for the prevention of tuberculosis. *Bull WHO* **1995**; *73:* 805–806.

6. World Health Organization. Joint statement. Consultation on human immunodeficiency virus (HIV) and routine childhood immunization. *Wkly Epidemiol Rec* **1987**; *62*: 297–299.

7. Aronson NE, Santosham M, Comstock GW, et al. Long term efficacy of BCG vaccine in American Indians and Alaska Natives: A 60-year follow-up study. *JAMA* **2004**; *291*: 2086–2091.

8. Black GF, Weir RE, Floyd S, et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. *Lancet* **2002**; *359*: 1393–1401

9. Brandt L, Feino Cunha J, Weinreich Olsen A, et al. Failure of the Mycobacterium bovis BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. *Infect Immun* **2002**; *70*: 672–678.

10. Waddell RD, Lishimpi K, von Reyn CF, et al. Bacteremia due to Mycobacterium tuberculosis or M. bovis, Bacille Calmette-Guerin (BCG) among HIV-positive children and adults in Zambia. *AIDS* **2001**; *15*: 55–60.

One of the various reasons proposed to explain the variable efficacy is the diversity of BCG strains. After many years of growth and passaging in the laboratory, divergent BCG vaccine strains have evolved, which differ from each other and from the original BCG. From1921, when BCG was first used, to 1961 when WHO recommended the lyophilisation and storage at -80°C for BCG cultures, vaccine BCG was sub cultured by numerous laboratories worldwide.

As a consequence, numerous variants appeared, including BCG Pasteur, BCG Moscow and BCG Brazil.[11] These various BCG strains are different in protection and effectiveness from each other and from their ancestors.[12]

BCG vaccines have been classified into two major groups. BCG Tokyo, Moreau, Russia and Sweden secrete a lot of MPB70, have two copies of the insertion sequence IS6110, and contain methoxymycolate and MPB64 genes. In contrast, BCG Pasteur, Copenhagen, Glaxo and Tice secrete little MPB70, have a single copy of the insertion sequence IS6110, and do not contain the methoxymycolate and MPB64 genes.[13]

Genomic comparisons have made it possible to determine the order of genetic events that include deletions and duplications, and changes in IS6110 copy number that occurred between its first use in 1921 and 1961. These complex genomic rearrangements in BCG strains have led to phenotypic and immunological differences, and possible variability in vaccine efficacy. Problems of sub-strain variability and protective efficacy of the current BCG vaccines could be overcome by new rationally constructed live vaccines, for which the attenuation factor and immunity are known.

11. Behr MA, Wilson MA, Gill WP, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science* **1999;** *284*: 1520–1523

12. Behr MA. BCG-different strains, different vaccines? *Lancet Infect Dis* **2002**; *2:* 86–92.

13. Ohara N, Yamada T. Recombinant BCG vaccines. *Vaccine* **2001**; *19*: 4089–4098

B.3 New Vaccines against Tuberculosis

Broadly, two approaches have been used to improve the TB vaccine. The first involves subunit vaccines. However, viable subunit vaccines that can deliver immunodominant mycobacterial antigens are yet to be developed.

Protein and DNA vaccines induce only partial protection against experimental TB infection in mice, and their efficacy is not better than that of BCG.[14] New antigen formulations, including multiple antigens or epitopes, are under investigation.[15, 16]

The second approach involves using live vaccines. These may be BCG strains that have been genetically manipulated to express immunodominant antigens, or attenuated strains of M. tuberculosis produced by random mutagenesis and targeted deletion of virulence genes.[17]

Subunit Vaccines

Potential TB subunit vaccines have been obtained by using immunodominant TB antigens and confer some degree of protection against M. tuberculosis in mice.[18] Protein fusions based on ESAT-6 and antigen 85B administered with a strong adjuvant to mice induce a dose-dependent immune response to the fusion proteins. This immune response is accompanied by protective immunity comparable to BCG-induced protection over a broad dose range that remained stable 30 weeks post-vaccination.[19]

14. Huygen K, Content J, Denis O, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nat Med* **1996**; *2:* 893–898.
15. Weinrich Olsen A, van Pinxteren LA, Meng Okkels L, Birk Rasmussen P, Andersen P. Protection of mice with a tuberculosis subunit vaccine based on a fusion protein of antigen 85b and esat-6. *Infect Immun* **2001**; *69*: 2773–2778.
16. Reed SG, Alderson MR, Dalemans W, Lobet Y, Skeiky YA. Prospects for a better vaccine against tuberculosis. *Tuberculosis (Edinb)* **2003;** *83*: 213–219.
17. Britton WJ, Palendira U. Improving vaccines against tuberculosis. *Immunol Cell Biol* **2003**; *81*: 34–45.
18. Olsen AW, Hansen PR, Holm A, Andersen P. Efficient protection against Mycobacterium tuberculosis by vaccination with a single subdominant epitope from the ESAT-6 antigen. *Eur J Immunol* **2000;** *30*: 1724–1732.
19. Weinrich Olsen A, van Pinxteren LA, Meng Okkels L, Birk Rasmussen P, Andersen P. Protection of mice with a tuberculosis subunit vaccine based on a fusion protein of antigen 85b and esat-6. *Infect Immun* **2001**; *69:* 2773–2778.

Selected M. Tuberculosis antigens have been used for the development of subunit vaccines against TB, for example Mtb72F in the AS02A formulation, which is the first recombinant TB vaccine to be tested in humans.[20]

Boosting BCG Vaccines

The Ankara recombinant modified vaccinia virus expressing M. tuberculosis Ag85A strongly boosts (BCG)-induced Ag85A specific CD4 (+) and CD8 (+) T-cell responses in mice.

The protection conferred correlated with the induction of Ag85A-specific, IFN-γ-secreting T cells in lung lymph nodes.[21]

Recombinant BCG Vaccine (rBCG)

Various strategies have been used to develop rBCG against mycobacteria diseases. One strategy is based on rBCG producing large amounts of autologous protective antigens that enhance immunity to other BCG antigens by increasing the expression of their genes. Recombinant BCG vaccine (rBCG30), also referred to as *a*-antigen and antigen Ag85B, expresses and secretes the 30-kDa major secreted protein of M. tuberculosis.[22]

Animals immunized with rBCG30 survived significantly longer than animals immunized with conventional BCG, when both groups were challenged with an aerosol of a highly virulent strain of M. tuberculosis.[23]

Alternatively, BCG genes that have been lost by deletion from parental M. bovis strain can be restored. An example is the case of ESAT-6 deleted from region RD1 of BCG.[24]

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20. Skeiky YA, Alderson MR, Ovendale PJ, et al. Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. *J Immunol* **2004**; *172*: 7618–7628.

21. Goonetilleke NP, McShane H, Hannan CM, Anderson RJ, Brookes RH, Hill AV. Enhanced immunogenicity and protective efficacy against Mycobacterium tuberculosis of bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara. *J Immunol* **2003**; *171*: 1602–1609.

22. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic S. Recombinant bacillus calmette-guerin (BCG) vaccines expressing the Mycobacterium tuberculosis 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc Natl Acad Sci USA* **2000**; *97*: 13853–13858.

23. Horwitz MA, Harth G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis. *Infect Immun* **2003**; *71*: 1672–1679.

A second strategy involves enhancement of the relatively low intrinsic ability of BCG to induce the CD8+ T-cell response.[25] Major histocompatability complex (MHC) class I-restricted CD8+ T cells are believed to play a major role in protection against mycobacterial infection. rBCG-secreting biologically active Hly that improves MHC class I-presentation of co-phagocytosed soluble protein have been constructed.[26]

In another approach rBCG have been constructed secreting diverse cytokines, including IL-2, IFN-γ and others.[27] This approach aims to enhance the immune stimulatory properties of BCG.

The possibility of rBCG as a vaccine vehicle capable of simultaneously expressing antigens of numerous pathogens is being explored.

Attenuated M. Tuberculosis as Live Vaccine

The advantage of attenuated M. tuberculosis strains as vaccines is that these strains still preserve the many hundreds of genes have been deleted from BCG as a consequence of the progressive adaptation of BCG strains to laboratory conditions.

Of the six immune-dominant antigens of M. bovis (ESAT-6, CFP10, Ag85, MPB64, MPB70, MPB83), five are either deleted from or down regulated in some or all BCG strains. The deletions include the immune-dominant antigens ESAT-6 and CFP10, which are important for protection against M. tuberculosis challenge in the guinea pig model.

24. Pym AS, Brodin P, Majlessi L, et al. Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. *Nat Med* **2003***; 9*: 533–539.

25. Kaufmann SH. Is the development of a new tuberculosis vaccine possible? *Nat Med* **2000; *6***: 955–960.

26. Hess J, Miko D, Catic A, Lehmensiek V, Russell DG, Kaufmann SH. Mycobacterium bovis Bacille Calmette-Guerin strains secreting listeriolysin of Listeria monocytogenes. *Proc Natl Acad Sci USA* **1998**; *95*: 5299–5304.

27. Ohara N, Yamada T. Recombinant BCG vaccines. *Vaccine* **2001**; *19*: 4089–4098.

A M. tuberculosis phoP mutant has been constructed by a single gene disruption[28] and is a promising candidate for vaccines. Auxotrophic mutants are attenuated to different degrees and have diverse potential as vaccine candidates as assessed in animal models.[29] However, there are major issues associated with the use of live organisms; in particular, ethical, safety and regulatory hurdles need to be overcome.

28. Perez E, Samper S, Bordas Y, Guilhot C, Gicquel B, Martin C. An essential role for phoP in Mycobacterium tuberculosis virulence. *Mol Microbiol* **2001**; *41*: 179–187.

29. Smith DA, Parish T, Stoker NG, Bancroft GJ. Characterization of auxotrophic mutants of Mycobacterium tuberculosis and their potential as vaccine candidates. *Infect Immun* **2001**; *69*: 1142–1150.

C. Rapid Diagnostic Tests for Tuberculosis

Rapid and accurate diagnosis is a very important element of health measures to control Tuberculosis. Current strategies aim to investigate patients presenting with clinical symptoms using a variety of diagnostic tests including radiology and microbiology, and establish the diagnosis. Specimen collection is a key element of investigations. For pulmonary TB, respiratory tract specimens are required. Expectorated sputum is thought to be the best specimen, but induced sputum, endotracheal aspiration, bronchial washings or aspirates taken during bronchoscopy, laryngeal swabs and gastric lavage may also be used. Other specimens include cerebrospinal, pericardial, synovial and ascitic fluids and blood, bone marrow, urine and fecal specimens.

C.1 Question of Interest

What are the diagnostic tests available for Tuberculosis?

Methodology

A systematic review of the scientific literature focusing on the diagnostic tests for tuberculosis was performed. The search covered The Cochrane Library, PUBMED, MEDLINE and EMBASE as the central databases and also covered the WHO database of publications. The search was performed using one or a combination of the following key words: tuberculosis, diagnosis, rapid tests, sensitivity, specificity, predictive values, evaluation.

Studies on adults or children with any form of tuberculosis were considered to be eligible for inclusion. No restriction was placed on study settings, and it included studies from any country. Randomized controlled trials were included for the review as randomized controlled trials are considered the highest level of evidence.

Only articles in the English language were included for review. The initial review of articles was followed by a review of the cross referenced articles. The related article link in PUBMED was used sequentially to identify other similar articles of interest.

C.2 Conventional Tests for the Diagnosis of Active TB

Microscopy

Microscopy is used to examine clinical specimens or cultures for the presence of Acid Fast Bacilli (AFB). The three most commonly used stains are Ziehl–Neelsen, auramine–rhodamine fluorochrome and Kinyoun stains.[30 ,31]

Microscopy indicates that AFB are present in the sample, but does not always indicate viable organisms *per se* or that the organism is *M. TB*. Approximately 40–50% of patients with pulmonary TB are smear- positive[32] (sputum must contain at least 5000 bacilli/ml for them to be detectable by microscopy). It is estimated that 10% of smear-negative patients are also culture negative. Patients with smear-negative, culture-positive TB appear to be responsible for about 17% of TB transmission.

Although microscopy is not very accurate, it remains the most rapid technique and helps identify the most infectious patients. In developing countries, microscopy is often the only test available for diagnosis of pulmonary TB. Sputum-negative cases are usually diagnosed on the basis of clinical and radiological indicators.[33]

Conventional Culture-Based Techniques

Culture is the most sensitive of currently available tests (sensitivity rates of up to 98% have been reported), and also permits identification and drug sensitivity tests to be made.

However, it may require up to 6–8 weeks for the isolation of *M. TB* from a clinical specimen and in 10–20% of cases the bacillus is not successfully cultured.[34]

30. Gordin F, Slutkin G. The validity of acid-fast smears in the diagnosis of pulmonary tuberculosis. *Arch Pathol Lab Med* **1990**;*114*: 1025–7.

31. Attorri S, Dunbar S, Clarridge JE, III. Assessment of morphology for rapid presumptive identification of *Mycobacterium tuberculosis* and *Mycobacterium kansasii*. *J Clin Microbiol* **2000**; *38*: 1426–9.

32. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* **1999**; *282*: 677–86.

33. Siddiqi K, Lambert ML, Walley J. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence. *Lancet Infect Dis* **2003**; *3*: 288–96.

34. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* **2000**; *356*: 1099–104

Traditionally, mycobacterium is grown on solid media, containing a cocktail of antimicrobial agents that permit only mycobacterium to replicate. The media are either egg-based [e.g. Lowenstein–Jensen (LJ) and Ogawa media] or agar-based (Middlebrook 7H9, 7H10 and 7H1177). When material from solid cultures is used, *M. TB* can often be distinguished from atypical mycobacterium. Culture is more expensive than microscopy and requires a high standard of technical competence.

Serological Tests

Numerous serological tests for TB exist, including complement fixation tests, haemagglutination tests, radioimmunoassays and enzyme-linked immunosorbent assays (ELISAs).[35] They have been extensively evaluated in developing countries, since they are less expensive, rapid and simple, making them ideal for use in resource-poor settings.[36] None of these tests have shown adequate accuracy and have not been widely implemented. Sensitivities of 16–57% and specificities of 62–100% have been reported.[37]

Antibody responses are directed against a broad set of antigens and responses vary individually. [38,39] It has been estimated that even 30% of patients with smear-positive pulmonary TB do not have detectable antibody to any single reagent.[40] Exposure to atypical mycobacteria, BCG vaccination and HIV prevalence influences results of serological tests further impacting accuracy.

Adenosine Deaminase and Cytokine Assays

35. Bothamley GH. Serological diagnosis of tuberculosis. *Eur Respir J Suppl* 1995;**20**:676s–88s.
36. Gounder C, Queiroz Mello FC, Conde MB, Bishai WR, Kritski AL, Chaisson RE, *et al*. Field evaluation of a rapid immunochromatographic test for tuberculosis. *J Clin Microbiol* **2002**; *40*: 1989–93.
37. Pottumarthy S, Wells VC, Morris AJ. A comparison of seven tests for serological diagnosis of tuberculosis. *J Clin Microbiol* **2000**; *38*: 2227–31.
38. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* **2000**; *356*: 1099–104.
39. Lyashchenko K, Colangeli R, Houde M, Al Jahdali H, Menzies D, Gennaro ML. Heterogeneous antibody responses in tuberculosis. *Infect Immun* **1998**; *66*: 3936–40.
40. Bothamley GH. Serological diagnosis of tuberculosis. *Eur Respir J Suppl* **1995**; *20*: 676s–88s

An enzyme produced by lymphocytes, adenosine deaminase (ADA), has been studied in the diagnosis of pleural TB, peritoneal TB and TB meningitis. Several cytokines, including interferon-γ and TNF-α, have been evaluated for the diagnosis of TB. As diagnostic tests, ADA and interferon-γ assays offer several advantages, they are rapid, simple, non-invasive (especially in diagnosis of pleural TB) and can be performed in most clinical laboratories. However both tests lack accuracy if used alone.

C.3 Rapid Diagnostic Tests for Active TB

Rapid Liquid Culture Systems

Faster culture of mycobacterial isolates has been achieved with manual culture systems [Septi-Chek AFB or the manual mycobacterial growth indicator tube (MGIT)].[41] Compared with automated systems, however, manual systems have clear disadvantages, including a longer time to detection of significant mycobacteria, more technical hands-on time and a higher contamination rate. The time to detection of growth of a mycobacterial species can be shortened significantly with the use of automated or semi-automated liquid culture systems. has been used for many years. Systems that rely on non-radiometric growth have been developed, because of increasing problems with handling and disposal of radioactive waste (as in the radiometric liquid culture using a broth of radio-labeled carbon), use of expensive media and staff time.[42] Nevertheless the semi-automated radiometric culture BACTEC 460TB system remains the fastest (14–17 days) and is widely accepted as a reference standard.[43]

Several non-radiometric automated or semi automated liquid culture systems that measure changes in gas pressure, carbon dioxide production or oxygen consumption fluorimetrically or colorimetrically are now available.

41. Sharp SE, Lemes M, Erlich SS, Poppiti RJ Jr. A comparison of the Bactec 9000MB system and the Septi-Chek AFB system for the detection of mycobacteria. *Diagn Microbiol Infect Dis* **1997**; *28*: 69–74

42. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *J Clin Microbiol* **2004**; *42*: 2321–5.

43. Tortoli E, Cichero P, Piersimoni C, Simonetti MT, Gesu G, Nista D. Use of BACTEC MGIT 960 for recovery of mycobacteria from clinical specimens: multicenter study. *J Clin Microbiol* **1999**; *37* :3578–82.

They allow continuous monitoring of cultures and there is no need for further operator input after loading the specimen. Major parameters in comparisons between these systems are recovery rates for mycobacteria, time to detection and contamination rates. Optimal recovery is usually achieved through a combination of rapid automated liquid culture systems and solid media.[44] Nevertheless three sputa still seem to be required for accurate diagnosis of TB.[45]

M. TB often exhibits a characteristic morphological pattern (serpentine cording) when grown in liquid media that has been used for rapid presumptive identification of *M. TB* and other mycobacterial species. The radiometric Bactec 460 and also the automated nonradiometric liquid culture systems also allow susceptibility testing. Reductions in turnaround times from 21 days for LJ-based tests to 6–12 days for automated liquid culture systems for susceptibility testing have been reported. The liquid culture methods are expensive, however, and require elaborate technology. In addition, skilled and experienced staff is crucial as contamination rates have been high when inexperienced and untrained staff used these systems.[46]

C.4 Methods for Rapid Detection and Identification Directly in Clinical Specimens

Nucleic Acid Amplification Tests

Nucleic acid amplification tests (NAATs) are molecular systems which are able to detect small amounts of genetic material (DNA or RNA target sequences) from the micro-organism, and are based on repetitive amplification of target sequences. If the target organism is not present in the sample, no amplification will occur. A variety of amplification methods may be used, including amplification of the target nucleic acid, such as the polymerase chain reaction (PCR), or amplification of a nucleic acid probe, such as a ligase chain reaction.

Polymerase Chain Reaction

44. Hanna BA, Ebrahimzadeh A, Elliott LB, Morgan MA, Novak SM, Rusch-Gerdes S, et al. Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. J Clin Microbiol **1999**; *37*: 748–52.

45. Harvell JD, Hadley WK, Ng VL. Increased sensitivity of the BACTEC 460 mycobacterial radiometric broth culture system does not decrease the number of respiratory specimens required for a definitive diagnosis of pulmonary tuberculosis. *J Clin Microbiol* **2000**; *38*: 3608–11

46. Drobniewski FA, Caws M, Gibson A, Young D. Modern laboratory diagnosis of tuberculosis. *Lancet Infect Dis* **2003**; *3*: 141–7.

PCR is the most common of these methods. The products from the PCR reaction are usually analyzed on an agarose gel. Detection of the amplified products can also be done by DNA sequencing, an enzyme immunoassay format using probe-based colorimetric detection or by fluorescence emission technology.[47]

The genetic material can be used to identify species and can sometimes be used to identify the genes that code antibiotic resistance. Although the specificity of a well-designed PCR can be high, the sensitivity is thought to be less than that of culture, but can be optimized by performing PCR on high-quality specimens. The sensitivity of a carefully performed quality controlled PCR would be expected to be 90–100% smear positive and 60–70% on smear-negative, culture-positive sputum samples.[48] Commercially available tests include the Roche Amplicor® *Mycobacterium tuberculosis* test[49] (PCR target amplification of part of the 16S rRNA gene, followed by colorimetric detection of the PCR product).

The Gen-Probe Amplified *Mycobacterium tuberculosi*s Direct Test (MTD®),[50] which is an isothermal transcription amplification method, uses rRNA as the target rather than DNA.

The BD ProbeTec (multiplex strand displacement system) also uses an isothermal amplification but with a DNA fragment.[51]

The majority of 'in house' PCR tests are based on the IS6110 insertion sequence owing to its presence in multiple copies in most isolates.

Using this technique as a diagnostic test can lead to PCR false positives, as mycobacteria other than TB contain this insertion element,[52] and to false negatives[53] as not all *M. TB* isolates contain a copy of the IS6110 element.

47. Louie M, Louie L, Simor AE. The role of DNA amplification technology in the diagnosis of infectious diseases. *CMAJ* **2000**; *163*: 301–9.

48. Drobniewski FA, Caws M, Gibson A, Young D. Modern laboratory diagnosis of tuberculosis. *Lancet Infect Dis* **2003**; *3*: 141–7.

49. American Thoracic Society Workshop. Rapid diagnostic tests for tuberculosis. What is the appropriate use? *Am J Respir Crit Care Med* **1997**; *155:* 1804–14.

50. Centers for Disease Control and Prevention. Nucleic acid amplification tests for tuberculosis. *MMWR Morb Mortal Wkly Rep* **1996**; *45*: 950–2.

51. McHugh TD, Pope CF, Ling CL, Patel S, Billington OJ, Gosling RD, *et al*. Prospective evaluation of BDProbeTec strand displacement amplification (SDA) system for diagnosis of tuberculosis in non-respiratory and respiratory samples. *J Med Microbiol* **2004**; *53*(Pt 12): 1215–19

In addition to IS6110, other target genes include MBP64, rpob and hsp65. PCR reduces the time for identification of *M. TB* and may be completed within 3–6 hours after the receipt of the specimen. PCR is not used routinely, especially in developing countries, in view of considerable cost and laboratory equipment and skills required.

However, PCR can be carried out on a crude extract directly from clinical samples; therefore, in resource-poor settings where culturing is difficult, PCR is often seen as an attractive alternative. Since PCR is able to detect small numbers of organisms, cross-contamination in busy clinical laboratories might yield significant numbers of false-positive results.

Ligase Chain Reaction

Ligase chain reaction (LCx® Tb test) is based on the amplification of a segment of the chromosomal gene of *M. TB* encoding for the protein antigen b. This gene sequence appears to be specific of the *M. TB* complex and has been detected in all *M. TB* complex strains examined to date.[54] High sensitivity and specificity of the test have been reported;[55] however the LCx® test has recently been withdrawn from the market owing to batch problems.

Loop Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method which relies on auto cycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment. The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops.

52. Kent L, McHugh TD, Billington O, Dale JW, Gillespie SH. Demonstration of homology between IS6110 of *Mycobacterium tuberculosis* and DNAs of other *Mycobacterium* spp. *J Clin Microbiol* **1995**; *33*: 2290–3.

53. El Dawi TG, Saeed eN, Hamid ME. Evaluation of a PCR-amplified IS6110 insertion element in the rapid diagnosis of pulmonary tuberculosis in comparison to microscopic methods in Sudan. *Saudi Med J* **2004**; *25*: 1644–7

54. Jouveshomme S, Cambau E, Trystram D, Szpytma M, Sougakoff W, Derenne JP, *et al*. Clinical utility of an amplification test based on ligase chain reaction in pulmonary tuberculosis. *Am J Respir Crit Care Med* **1998**; *158*: 1096–101

55. Fadda G, Ardito F, Sanguinetti M, Posteraro B, Ortona L, Chezzi C, *et al*. Evaluation of the Abbott LCx *Mycobacterium tuberculosis* assay in comparison with culture methods in selected Italian patients. *New Microbiol* **1998**; *21***:** 97–103

LAMP has the following characteristics: (i) all reactions can be conducted under isothermal conditions ranging from 60 to 65°C by using only one type of enzyme; (ii) the specificity of the reaction is extremely high because it uses four primers recognizing six distinct regions on the target DNA; (iii) amplification can be performed in a shorter time than amplification by PCR because there is no time loss due to thermal cycling; and (iv) it produces extremely large amounts of amplified products and enables simple detection methods such as visual judgment by the turbidity or fluorescence of the reaction mixture, which is kept in the reaction tube.[56] LAMP of DNA is a powerful tool to facilitate point-of-care genetic testing at the bedside. When two more primers, termed loop primers, were added, the LAMP reaction time could be even less than half of that for the original LAMP method.[57] In this procedure, six primers recognize eight distinct regions on the targeted DNA.

The LAMP reaction with a 60-min incubation and visual inspection has a sensitivity equivalent to that of the Amplicor test. Both methods showed a detection limit of 5 to 50 genomes per test for the three mycobacterial species.

The sensitivity of the LAMP assay was 10 to 100 times lower when the reaction time was shortened to 35 min.[58]

A prototype LAMP assay with simplified manual DNA extraction was evaluated for accuracy and ease-of-use in a clinical evaluation of LAMP for the detection of pulmonary TB in microscopy centers in Peru, Bangladesh and Tanzania, to determine its operational applicability in such settings.[59] The sensitivity of LAMP in smear and culture-positive sputum specimens was 97.7% (173/177 specimens, 95% confidence interval (CI) 95.5 - 99.9%) and in smear-negative, culture-positive specimens 48.8% (21/43 specimens, CI 33.9 - 63.7%).

56. Mori, Y., K. Nagamine, N. Tomita, and T. Notomi, Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem. Biophys. Res. Commun.* **2001;** *289*: 150–154.

57. Nagamine, K., T. Hase, and T. Notomi, Accelerated reaction by loop mediated isothermal amplification using loop primers. *Mol. Cell. Probes* **2002**; *16:* 223–229.

58. Iwamoto T, Sonobe T, Hayashi K. Loop-Mediated Isothermal Amplification for Direct Detection of *Mycobacterium tuberculosis* Complex, *M. avium*, and *M. intracellulare* in Sputum Samples. *Journal of Clinical Microbiology* **2003;** *41(*6): 2616–2622

59. Boehme CC, Nabeta P, Henastroza G, Raqib R, Rahim Z, Gerhardt M, Sanga E, Hoelscher M, Notomi T, Hase T, Perkins MD. Operational feasibility of using loop-mediated isothermal amplification (LAMP) for the diagnosis of pulmonary TB in microscopy centers of developing countries. *J Clin Microbiol*. **2007** Mar 28; [Epub ahead of print]

The specificity in culture-negative samples was 99% (500/505 specimens, CI 98.1 - 99.9%). The average hands-on time for testing 6 samples and 2 controls was 54 minutes, similar to that of sputum smear microscopy. The optimal amplification time was 40 minutes. No indeterminate results were reported and the inter-reader variability was 0.4%. Despite the use of a simple room for all procedures, no DNA contamination was observed. The assay was robust, with high end-point stability and low rate of test failure. Technicians with no prior molecular experience easily performed the assay after 1 week of training, and opportunities for further simplification of the assay were identified.

C.5 Mycobacteriophage-Based Methods

Mycobacteriophage tests are an alternative to PCR tests and may be useful for resource-poor countries where PCR is impractical. Rapid phenotypic-based methods have been applied directly to clinical specimens, although greater success has been achieved with cultured isolates.[60] These tests have the advantage of being easy to perform and present a low-cost means to screen for antimicrobial resistance.[61] Mycobacterial cultures are infected with mycobacteriophage and exogenous, non-infecting phage is killed. The signal is amplified biologically by replication of the phage within mycobacteria and detected normally by one of two methods. The simplest of these is the phage amplified biological (PhaB) assay, where the phage is plated on to a lawn of the rapidly growing *M. smegmatis*, which is also lysed by the phage and a numerical result is obtained relating to the number of viable mycobacteria in the original sample.[62]

Alternatively, a luciferase reporter phage may be used. When infecting viable mycobacteria, it produces quantifiable light that is not observed if drug-sensitive mycobacteria are rendered nonviable by treatment with active antimicrobials. Light can be detected with a Polaroid film box.

60. Watterson SA, Drobniewski FA. Modern laboratory diagnosis of mycobacterial infections. *J Clin Pathol* **2000**; *53:* 727–32

61. Eltringham IJ, Wilson SM, Drobniewski FA. Evaluation of a bacteriophage-based assay (phage amplified biologically assay) as a rapid screen for resistance to isoniazid, ethambutol, streptomycin, pyrazinamide, and ciprofloxacin among clinical isolates of Mycobacterium tuberculosis. *J Clin Microbiol* **1999**; *37*: 3528–32.

62. Wilson SM, al Suwaidi Z, McNerney R, Porter J, Drobniewski F. Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of *Mycobacterium tuberculosis*. *Nat Med* **1997**; *3*: 465–8.

Either of these methods can also be used to determine drug resistance by incubating the culture with the relevant antibiotic, as only viable mycobacteria will be detected by the phage assay.

C.6 Methods for the Rapid Identification of Mycobacterial Species from Cultured Isolates and Drug Susceptibility Testing

High-Performance Liquid Chromatography (HPLC)

HPLC is used to analyze mycolic acids extracted from an unknown organism with ultraviolet or fluorescence detection: the HPLC pattern is compared with a library of known patterns, usually facilitated by a decision analysis system. HPLC methods have shown high sensitivity and specificity. However, high equipment costs and the level of expertise required for the analysis have restricted its use.[63]

Bactec NAP Test

NAP-selective inhibition of *M. TB* complex is a conventional biochemical test used in species identification that has been adapted for use with the radiometric Bactec system.[64] *M. TB* and *M. bovis* are both susceptible to NAP (a chloramphenicol-related compound that inhibits growth), whereas atypical mycobacteria are resistant to it.[65] The Bactec radiometric growth system can be used for rapid presumptive identification of *M. TB* where NAP is used along with a growth control tube.

Nucleic Acid Probes

A nucleic acid probe, such as the AccuProbe *M. TB,* has significantly reduced the time required to isolate and identify *M. TB*.[66]

63. Butler WR, Guthertz LS. Mycolic acid analysis by high-performance liquid chromatography for identification of *Mycobacterium* species. *Clin Microbiol Rev* **2001**;*14*:704–26.

64. Eisenstadt J, Hall GS. Microbiology and classification of mycobacteria. *Clin Dermatol* **1995**; *13*: 197–206.

65. Anargyros P, Astill DS, Lim IS. Comparison of improved BACTEC and Lowenstein–Jensen media for culture of mycobacteria from clinical specimens. *J Clin Microbiol* **1990**; *28*: 1288–91.

66. Hale YM, Desmond EP, Jost KC Jr, Salfinger M. Access to newer laboratory procedures: a call for action. *Int J Tuberc Lung Dis* **2000**; *4*(12 Suppl 2): S171–5.

The AccuProbe system can be used for the identification of *M. TB* complex, *M. avium complex*, *M. avium*, *M. intracellulare*, *M. kansasii* and *M. gordonae* from culture. The bacterial membranes are lysed, releasing the DNA into solution. DNA probes complementary to the bacterial target sequence, specific to the organism being identified, are used to identify whether a culture, once grown, is *M. TB* or atypical.

The AccuProbe is rapid and simple to perform and takes about 1–2 hours from culture. Although certain of the more clinically significant atypical mycobacteria can be identified, such as *M. avium complex*, a separate test must be performed for each species which is tested for.

Other rapid identification systems include the LIPA mycobacteria kit, which is a PCR-based reverse hybridization line probe assay. The Inno-LIPA Rif TB identifies 95% of RMP-resistant isolates. Both Accuprobe and LIPA mycobacteria tests have been reported to have accuracies above 90%, but the cost of such probes limits their routine diagnostic use.

Nucleic Acid Amplification-based Methods and DNA Sequencing

In developed countries, PCR-based assays are often routinely used to identify cultures, such as the Hain DNA strip for the identification of *M. TB* complex and non-tuberculosis mycobacteria (NTM) strains125 or the INNO-LIPA assay for *M. TB* identification and rifampicin resistance. Direct detection of *M. TB* in respiratory specimens by DNA sequencing is used to identify drug resistance. RMP resistance is important, since it is often a surrogate for MDR-TB. About 95% of all *M. TB* RMP-resistant clinical isolates harbor specific mutations within a region of the rpoB gene. In contrast to RMP, genotypic testing for INH resistance is much more complex and alterations in several genes including *katG* and *inhA* have been reported. This technique uses high-cost equipment beyond the reach of most clinical laboratories except at the reference level.

PCR Restriction Enzyme Analysis

PCR restriction enzyme analysis (PRA) is based on the PCR amplification of a fragment of the *hsp65* gene, followed by restriction. It is a simple and rapid identification method and the turnaround time may be 24–48 hours. PRA seems to be an efficient method for the identification of mycobacteria to the species level.

High accuracy of this method has been reported at relatively low cost compared with, for example, identification of *M. TB* isolates with the Accuprobe.

C.7 Tests for the Detection of Latent TB Infections (LTBI)

Tuberculin Skin Test (TST)

TST is currently the standard tool to detect latent TB infection, although it is far from a 'gold' standard. TST is based on the detection of delayed-type hypersensitivity to purified protein derivative (PPD), a mixture of antigens shared by several mycobacteria that gives rise to a skin reaction. Two visits are required for the test, one for PPD inoculation (the Mantoux technique uses intracutaneous injection by needle and syringe) and another after 48–72 hours for interpretation of the result based on the size of the skin reaction.

TST is relatively cheap and can be performed without the need for a specialist laboratory. Difficulties in test administration and interpretation often lead to false results. Dose of PPD, method of application and criteria for interpretation vary between countries. Weak PPD doses increase the likelihood of false-negative results and strong doses increase the likelihood of false-positive results.

A 1.5-mm difference of reaction size may be seen when a 10-tuberculin unit (TU) dose is compared with a 5-TU dose. The technique for inoculating PPD doses may cause false results. For example, the Heaf test, used in the UK, is usually less precise than the Mantoux test, although the two tests generally correlate. Different cut-offs are used for positivity of TST, as there is no general consensus on this issue. Criteria of 5, 10 or 15 mm for skin reaction have been recommended depending on the clinical situation. There can be false TST results from operator variability in both inoculation and reading of the test. Digit preference, for example rounding measures of TST induration to the nearest multiple of 5 mm and interpretation bias, can significantly affect TST results.

There are many reasons for false-positive TST results. PPD contains a poorly defined mixture of mycobacterial antigens. Because antigens are shared with other mycobacteria, tuberculin reactivity leading to a positive TST can result from BCG vaccination with a live attenuated mycobacterial strain derived from *M. bovis* or from exposure to atypical mycobacteria. The effect of BCG vaccination on TST can persist as long as 15 years after vaccination.

Reaction due to BCG vaccination tends to be small, but this is not always consistent. Specificity problems of PPD can be addressed by simultaneous skin testing with *M. TB* PPD and sensitins, which are PPD-like products derived from atypical mycobacteria. This approach can help to discriminate patients with TB from those who are infected with *M. avium complex*. Repeated TSTs may induce booster responses leading to false-positive results.

Anergy associated with HIV infection, disseminated TB or immunosuppression due to haemodialysis, transplantation or medication can give rise to false-negative reactions. There have been many responses to the above problems, none of which are ideal. Some countries have stopped using either BCG vaccination altogether, or school-age vaccination, as BCG has only limited effect on preventing adult tuberculosis. An alternative strategy (and the policy of the USA and The Netherlands) has been to use TST to identify recently infected individuals and give isoniazid chemoprophylaxis. The British Thoracic Society no longer recommends performing TST among BCG-vaccinated people with recent TB exposure.

Immune-based Blood Tests: The Interferon-γ Assays

Interferon- γ assays have been developed as tests to replace TST. Blood samples are taken from the patient and incubated with mycobacterial antigens specific for *M. TB* complex strains and absent from the BCG vaccine strain. T lymphocytes within the blood sample produce interferon- γ as a marker of infection or active TB.[67] Since *M. TB* is an intracellular pathogen, assessment of whether a patient's T cells have been exposed to and sensitized by antigens specific to *M. TB*, may provide an alternative approach to diagnosis.[68]

The antigens used to elicit an interferon- γ response define the two main types of the available tests: assays based on PPD and those based on RD1-specific antigens including early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). Various commercial and in-house tests based on PPD, ESAT-6 and/or CFP-10 has been evaluated using either an enzyme-linked immunospot assay (ELISPOT) or an enzyme-linked immunosorbent assay (ELISA).

67. Lein AD, von Reyn CF. *In vitro* cellular and cytokine responses to mycobacterial antigens: application to diagnosis of tuberculosis infection and assessment of response to mycobacterial vaccines. *Am J Med Sci* **1997**; *313*: 364–71.

68. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* **2000**; ***356***: 1099–104.

The two commercial tests using ELISA are Quantiferon® (based on PPD) and Quantiferon Gold®150 (based on ESAT-6 and CFP-10). The T SPOT-TB® assay is an ELISPOT assay and is also based on RD1-specific antigens.

Interferon- γ assays have several advantages over TST. They involve having a blood test at a single visit and a return visit might not be needed in some settings, depending on the test result. Automated testing has the advantage of reducing reader bias as interpretation is objective.

A booster phenomenon does not occur and therefore screening of people who are repeatedly exposed to TB (e.g. healthcare workers) becomes feasible. Interferon- γ assays might improve diagnostic accuracy in latently infected people with greatest risk of progression in whom TST is often false negative, e.g. people with HIV infection.

Interferon- γ assays also have some limitations. The need to perform a blood test might not be desirable to certain patient groups. The blood often needs to be processed within 12 hours after collection and laboratories need to gain expertise in technology like isolation of mononuclear cells.

The Quantiferon test measures interferon- γ production after *in vitro* stimulation of whole blood cells with PPD from *M. TB* and control antigens. It is able to discriminate between *M. TB* and *M. avium intracellulare complex* infection. It responds to multiple antigens spontaneously. It does not boost anamnestic immune responses. Two key disadvantages of Quantiferon are that it can give false-positive results in BCG-vaccinated people and that it does not discriminate between most of the atypical mycobacteria and *M. TB*.

Interferon- γ assay based on RD1-specific antigens, ESAT-6 and CFP-10, can overcome some of the above disadvantages. Comparative genomics has identified several genetic regions in *M. TB* and *M. bovis* that are deleted in all tested BCG strains. The identified region, so called RD1 region, is present in *M. kansasii*, *M. szulgai* and *M. marinum*. Proteins encoded in these regions have formed the basis of new specific T cell-based blood tests that do not cross-react with BCG, but only two antigens, ESAT-6 and CFP 10, have been studied in detail in humans. All stimulated T-lymphocytes secrete interferon- γ, but the ESAT-6- and CFP-10-specific assays can only detect interferon- γ secreted from T-lymphocytes produced as a result of exposure to ESAT-6 and CFP-10 antigen.

In vivo and *in vitro* experiments have shown that the combination of ESAT-6 and CFP-10 has a higher sensitivity and specificity than PPD in diagnosis of TB infection.

Use of Interferon-Gamma Assays for Active TB Infection

Interferon- γ assays are being assessed for use in people with suspected active TB infection. The key problem with their use in this context is that they detect LTBI and not only active disease. Where the prevalence of TB is low and clinical indications are strong, a positive test result may be assumed to indicate active TB. However, in TB endemic countries a positive test result would be less meaningful, potentially indicating only latent TB infection. However, since *M. TB* infection is a necessary prerequisite for active TB disease, a negative test result can effectively exclude a diagnosis of TB, if the test has sufficiently high diagnostic sensitivity. These tests could potentially serve as useful 'rule-out' tests in patients with suspected TB in low and high prevalence countries.

C.8 Accuracy of the RDTs[69]

NAAT Tests

NAAT test accuracy was far superior when applied to respiratory samples as opposed to other specimens. Although the results were not statistically significant, the AMTD test appears to perform better than other currently available commercial tests. The better quality in-house studies were, for pulmonary TB, much better at ruling out TB than the commercial tests (higher sensitivity), but were less good at ruling it in (lower specificity). Given that these tests are in no way standardized and cover a wide range of different target genes and procedures, it is not possible to recommend any one over another owing to a lack of direct test comparisons. The specificity of NAAT tests is high when applied to body fluids but sensitivity is almost uniformly poor, indicating that these tests cannot be used reliably to rule out TB but are of value in 'ruling in' a diagnosis with compatible clinical and/or radiological findings. The main problem appears to be with the sensitivity of the tests, presumably due to difficulties in obtaining fluid samples with sufficient mycobacteria to allow amplification.

69. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, *et al*. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess* **2007**; *11(3)*.

High specificity estimates suggest that NAAT tests should in fact be the first-line test for ruling in TB meningitis, but that they need to be combined with the results of other tests in order to rule out disease.

Phage Tests

Although the body of evidence is small, the phage tests have been shown to have high specificity in pulmonary TB but it is not clear whether these tests have sufficiently high sensitivity in smear-negative samples to recommend their routine use in practice. They do have the potential advantage over NAAT tests that they detect only viable mycobacteria. Phage and some forms of NAAT are able to yield antibiotic sensitivities within 2–3 days.

Adenosine Deaminase Tests

There is no evidence to support the use of ADA tests for the diagnosis of pulmonary TB. However, there is considerable evidence to support their use in pleural fluid samples for diagnosis of pleural TB, where sensitivity was very high, and to a slightly lesser extent for TB meningitis. In both pleural TB and TB meningitis, ADA tests had higher sensitivity than any other tests. Further research is required to determine the specificity of this test with regard to non-tuberculosis pleural effusions of infectious or inflammatory etiology. Of all the tests evaluated for peritoneal or pericardial TB, ADA appeared the most promising; however, further research is needed.

Interferon-γ

The assessment of interferon-γ levels in pleural fluid samples is very promising for the detection of pleural TB.

Diagnostic sensitivity was high, but further studies, including patients with infectious and inflammatory non-tuberculosis pleural effusions, are required to determine the likely specificity of these tests in routine practice for evaluating patients with suspected pleural TB.

Anti-TB Antibody Tests

Serum anti-TB antibody test performance was universally poor, regardless of type of TB, although some studies did show high specificity.

Fully Automated Liquid Culture

Fully automated liquid culture methods are superior to culture on solid media in terms of their speed and their precision for the detection of isolates. Although the BACTEC 460 radiometric method has the same benefits, it is radiometric and therefore requires disposal of radioactive waste and also requires more staff time compared with the fully automated methods. The fully automated methods also had higher contamination rates than the BACTEC 460, but data on contamination rates may be somewhat outdated, as recent developments including the addition of antibiotics to the specimen vials appear to have reduced contamination. It is not certain if antibiotic addition increased the time-to-culture for specimens.

Interferon-γ Assays for Detection of Latent TB Infection

Interferon-γ blood tests based on RD1-specific antigens, ESAT-6 or CFP-10, correlate better with intensity of TB exposure, and therefore are more likely to detect LTBI accurately, than TST- and PPD-based assays. An additional advantage is that they are more likely to be independent of BCG vaccination status (owing to higher specificity as the key proteins are not produced by BCG) and HIV status (owing to higher sensitivity).

Table 1: Accuracy of Rapid Diagnostic Tests for Tuberculosis

Test	Sensitivity	Specificity	Benefit/Limitation
NAAT tests	High for pulmonary TB Low for other forms	Low for pulmonary TB High for other forms	Wide range of tests, Standardization an issue
Phage tests	High Sensitivity	High specificity for pulmonary TB	Detect only viable mycobacteria, sensitivity unclear for smear negative samples
Adenosine deaminase tests	High sensitivity for extra pulmonary TB	Specificity to be tested further	Does not show good results for pulmonary TB
Interferon- γ	High sensitivity for extra pulmonary pleural TB	Specificity to be tested further	Assays under development for detection of latent TB (will be independent of BCG vaccine and HIV status)
Anti-TB antibody tests	Poor sensitivity	Poor specificity	Currently, not recommended
Fully automated liquid culture tests	High	High	Rapid turnaround of results compared to conventional culture methods, Cost a limitation

D. Novel Drug Delivery Systems for Tuberculosis

D.1 Question of Interest

What are the treatment strategies for tuberculosis?

Methodology

A systematic review of the scientific literature focused on the treatment strategies for tuberculosis was performed. The search covered The Cochrane Library, PUBMED, MEDLINE and EMBASE as the central databases and also covered the WHO database of publications. The search was performed using one or a combination of the following key words: treatment, tuberculosis, coverage, evaluation, impact, direct observed therapy, drug delivery.

Studies on adults or children with any form of tuberculosis and lab based studies were considered to be eligible for inclusion. No restriction was placed on study settings and it included studies from any country. Randomized controlled trials were included for the review as randomized controlled trials are considered the highest level of evidence.

Only articles in the English language were included for review. The initial review of articles was followed by a review of the cross referenced articles. The related article link in PUBMED was used sequentially to identify other similar articles of interest.

D.2 Current Anti-Tuberculosis Chemotherapy

The WHO recommends treatment of TB and drug resistant cases with multi-drug therapy comprising:

1. An initial intensive phase of rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ), and ethambutol (ETB) daily for 2 months.

2. A continuation phase of RIF and INH for a further 4 months, either daily or 3 times per week.

INH eradicates most of the rapidly replicating bacilli in the first 2 weeks of treatment, together with streptomycin and ETB.

Thereafter, RIF and PYZ have an important role in the sterilization of lesions by eradicating organisms; these two drugs are crucial for successful 6-month treatment regimens.

RIF kills low or non-replicating organisms and the high sterilizing effect of PYZ serves to act on semi-dormant bacilli not affected by any other anti-TB agents in sites hostile to the penetration and action of the other drugs. INH and RIF, the two most potent anti-TB drugs, kill more than 99% of tubercular bacilli within 2 months of initiation of therapy. Using these drugs in conjunction with each other reduces anti-TB therapy from 18 months to 6 months.

The emergence of strains resistant to either of these drugs causes major concern, as treatment is then deferred to drugs that are less effective, have more toxic side effects, and result in higher death rates, especially among HIV-infected persons. TB is treated with a multi-drug regimen, and is thus exceptionally vulnerable to incidences of side effects, unsatisfactory patient compliance and slow improvement of patients.

Therefore, despite the availability of these highly effective treatments for TB, cure rates remain low, as commercial anti-TB formulations are inconvenient to administer and patients do not take the prescribed medications with sufficient regularity and duration to achieve a cure. Patients have to consume a large number of tablets (up to eight at one time), which is a common cause for non-compliance. It can be anticipated that non-optimal application of these short course regimens will result in the deterioration of their therapeutic potential, an escalation in the mortality rate and increased risk of developing acquired drug resistance. Resistance of *M. tuberculosis* to anti-TB agents is a worldwide problem in both immune-competent and HIV-infected populations.

D.3 Novel Drug Delivery Systems

Drug delivery, which takes into consideration the carrier, the route and the target, has evolved into a strategy of processes or devices designed to enhance the efficacy of therapeutic agents through modified or controlled release.

This may involve enhanced bioavailability, improved therapeutic index, or improved patient acceptance or compliance.

Drug delivery has been defined [70]as 'the use of whatever means possible, be it chemical, physicochemical or mechanical, to regulate a drug's access rate to the body's central compartment, or in some cases, directly to the involved tissues'.

To minimize toxicity and improve patients' compliance, extensive progressive efforts have been made to develop various implants, micro particulates, and various other carrier-based drug delivery systems to either target the site of *M. tuberculosis* infection or reduce the dosing frequency, which forms an important therapeutic strategy to improve patient outcomes.[71,72] Recent trends in controlled drug delivery have seen microencapsulation of pharmaceutical substances in biodegradable polymers as an emerging technology. Carrier or delivery systems such as liposomes and microspheres have been developed for the sustained delivery of anti-TB drugs and have demonstrated better chemotherapeutic efficacy when investigated in animal models (e.g. mice). Anti-TB drugs have been successfully entrapped and delivered in biodegradable polymers such as poly (DL-lactide- co-glycolide) (PLG), which are biocompatible and release drug in a controlled manner at therapeutic levels.[73,74,75]

When injected subcutaneously as a single dose, the micro particles, having a diameter ranging from 11.75 µm to 71.95 µm, provided sustained release of drugs over 6–7 weeks when tested in mice. The authors previously observed that particles with a size range >10 µm remained at the site of injection forming a depot.

70. Flynn GL: Considerations in controlled release drug delivery system. *Pharm Tech* **1982**; *6*: 33-39.

71. Piney R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B: Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. *J Antimicrob Chemother* **2003**, *52*: 981-986.

72. Falk R, Randolph TW, Meyer JD, Kelly RM, Manning MC: Controlled release of ionic compounds from poly (L-lactide) microspheres produced by precipitation with a compressed antisolvent. *J Contr Rel* **1997**, *44(1)*: 77-85.

73. Dutt M, Khuller GK: Chemotherapy of Mycobacterium tuberculosis infections in mice with a combination of isoniazid and rifampicin entrapped in Poly (DL-lactide-co-glycolide) micro particles. *J Antimicrob Chemother* **2001**, *47(6):* 829-35.

74. Dutt M, Khuller GK: Sustained release of isoniazid from a single injectable dose of poly (DL-lactide-co-glycolide) micro particles as a therapeutic approach towards tuberculosis. *Int J Antimicrob Agents* **2001**, *17*: 115-122

75. Kailasam S, Daneluzzi D, Gangadharam PRJ: Maintenance of therapeutically active levels of isoniazid for prolonged periods in rabbits after a single implant of biodegradable polymer. *Tuber Lung Dis* **1994**, *75(5):* 361-365.

The entrapped contents of the micro particles were gradually released by diffusion through the polymeric particles. Such depots can show release profiles extending over several months culminating in degradation of the entire polymeric device.

However, these formulations have to be injected either subcutaneously or intravenously, and the pain and discomfort associated with these routes of administration, in general, may often be unacceptable.

On oral administration of drug-loaded nanoparticles to *M. tuberculosis* infected mice at every 10th day, no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment. Therefore, oral nanoparticles-based anti-TB drug therapy can allow for a reduction in dosing frequency for better management of TB.[76] An osmotically regulated capsular multi-drug oral delivery system comprising asymmetric membrane coating- and dense semi permeable membrane coating-capsular systems for the simultaneous controlled administration of RIF and INH for the treatment of TB has been developed.[77] The modified asymmetric system provided satisfactory sustained release of RIF and INH, with an initial burst release that may be sufficient to achieve minimum effective concentration in blood. Thereafter, the system provided the release of the drugs in a near zero order rate – an ideal release profile for controlled drug delivery. In turn, this improved the safety profile of the drugs and enhanced the activity duration of drugs exhibiting short half-lives. The once daily system is optimal, and could potentially enhance patient compliance. In addition to these combinations, the development of a number of RIF-only controlled release formulations for the improvement of the clinical efficacy of the drug and patient compliance is in process.

Further attempts to solve the problems inherent in multidrug therapy have included the development of biodegradable polymeric micro- or nanoparticulate carrier systems to target alveolar macrophages that harbor *M. tuberculosis*.[78,79,80]

76. Ain Q, Sharma S, Garg SK, Khuller GK: Role of poly [DL-lactideco-glycolide] in development of a sustained oral delivery system for antitubercular drug(s). *Int J Pharm* **2002**, *239(1-2):* 37-46.

77. Prabakaran D, Singh P, Jaganathan KS, Vyas SP: Osmotically regulated asymmetric capsular systems for simultaneous sustained delivery of anti-tubercular drugs. *J Contr Rel* **2004**; *95(2):* 239-248.

78. Ahsan F, Rivas IP, Khan MA, Torres Suarez AI: Targeting to macrophages: role of physicochemical properties of particulate carriers-liposomes and microspheres-on the phagocytosis by macrophages. *J Contemp Relig* **2002**, *79*: 29-40.

79. Makino K, Nakajima T, Shikamura M, Ito F, Ando S, Kochi C, Inagawa H, Soma G, Terada H: Efficient intracellular delivery of rifampicin to alveolar macrophages using rifampicin-loaded PLGA

In the case of pulmonary TB, delivering the drug directly to the site of infection through inhalation of an aerosolized delivery system has the inherent advantages of bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects.[81]

There are pharmacokinetic and chemotherapeutic studies with aerosolized alginate nanoparticles encapsulating INH, RIF and PZA and RIF, INH, PYZ, ETB. [82,83] The nanoparticles were prepared by cation-induced gelification of alginate and were 235.5 ± 0 nm in size, with drug encapsulation efficiencies of 70– 90% for INH and PZA and 80–90% for RIF and 88–95% for EMB. The majority of particles (80.5%) were in the respirable range, with a mass median aerodynamic diameter of 1.1 ± 0.4 μm and geometric standard deviation of 1.71 ± 0.1 μm. The chemotherapeutic efficacy of three doses of drug-loaded alginate nanoparticles nebulised 15 days apart was comparable with 45 daily doses of oral free drugs. Thus, inhalable alginate nanoparticles could potentially serve as an ideal carrier for the controlled release of anti-TB drugs. This system has to be evaluated before use in humans.

The chemotherapeutic potential of nebulised solid lipid nanoparticles (SLNs) incorporating RIF, INH and PYZ against experimental TB has been evaluated.[84] SLNs are nanocrystalline suspensions in water, prepared from lipids, which are solid at room temperature. The SLNs, prepared by the emulsion solvent diffusion technique, possessed a favorable mass median aerodynamic diameter suitable for bronchoalveolar drug delivery.

microspheres: effects of molecular weight and composition of PLGA on release of rifampicin. *Colloids Surf, B: Biointerfaces* **2004**, *36*: 35-42.

80. Dutt M, Khuller GK: Chemotherapy of Mycobacterium tuberculosis infections in mice with a combination of isoniazid and rifampicin entrapped in Poly (DL-lactide-co-glycolide) micro particles. *J Antimicrob Chemother* **2001**, *47(6)*: 829-35.

81. Zhou H, Zhang Y, Biggs DL, Manning MC, Randolph TW, Christians U, Hybertson BM, Ng K: Microparticle-based lung delivery of INH decreases INH metabolism and targets alveolar macrophages. *J Contr Rel* **2005**, *107(2)*: 288-299.

82. Zahoor A, Pandey R, Sharma S, Khuller GK: Pharmacokinetic and pharmacodynamic behaviour of antitubercular drugs encapsulated in alginate nanoparticles at two doses. *Int J Antimicrob Agents* **2006**, *27(5):* 409-416

83. Zahoor A, Sharma S, Khuller GK: Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. *Int J Antimicrob Agents* **2005**, *26(4):* 298-303.

84. Pandey R, Khuller GK: Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. *Tuberculosis* **2005**, *85(4)*: 227-234

Following a single nebulisation to guinea pigs, therapeutic drug concentrations were maintained in the plasma for 5 days and in the organs for 7 days whereas free drugs were cleared after 1–2 days.

Liposomes coated with alveolar macrophage- specific ligands demonstrated preferential accumulation in alveolar macrophages, maintaining high concentrations of RIF in the lungs even after 24 hours.

Studies have demonstrated that a single implant of INH in polylactic-co-glycolic acid (PLGA) copolymer could ensure sustained levels of free INH for a period of up to 8 weeks following implantation in rabbits. Single implants of INH and PYZ in mice have also been tested. Such devices, however, inherently suffer from the disadvantages of immobilization at the implantation site and surgical requirements for implantation.

The WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) encourage use of Fixed Dose Combination (FDC) formulations – this evolved from the fact that TB always requires multi-drug treatment. Patients should thus be given FDC adjusted for body weight whenever self-administration of anti-TB drugs is permitted. A FDC – which is a combination of two or more first-line anti-TB drugs in a single formulation at a fixed proportion – prevents monotherapy; and it is expected that this will reduce the emergence of MDR TB; simplify treatment, and thus minimize prescription error and increase patient and doctor compliance; simplify drug stock management, shipping and distribution; and reduce the risk of misuse of RIF for conditions other than TB. A major concern is the issue of unacceptable RIF bioavailability in a number of FDC anti-TB formulations.

The decomposition of RIF has varied from 8.5 to 50% in the acidic environment of the stomach in the time range corresponding to the gastric residence time for most dosage forms in humans (≈15 minutes to 105 ± 45 minutes). However, the gastric-emptying time for some single-unit dosage forms may reach 6 hours. The factors proposed for this variation in the bioavailability of RIF from different FDC formulations include the particle size and crystalline form of the drug, and the manufacturing process. The effect of these factors, however, has not been convincingly explained in previous studies. RIF is known to undergo hydrolysis in acidic medium to the insoluble 3-formyl rifamycin SV (3 FRSV). INH accelerates degradation of RIF into this poorly absorbed derivative (3 FRSV) in the acidic environment of the stomach via reversible formation of the isonicotinyl hydrazone of 3-FRSV with INH.

RIF in the presence of INH as a FDC may undergo greater decomposition in the acidic conditions of the stomach, as compared to when RIF is administered (orally) alone. Thus, less RIF will be available for absorption from FDCs as compared to RIF administered as a separate formulation. This will be reflected in the poor bioavailability from the former formulation.

There is thus an urgent need to modify or segregate the FDC formulation in such a way that RIF and INH are not released simultaneously in the stomach. Alternatively both drugs need to be administered separately after an interval corresponding to average gastric residence time, which is somewhat unpredictable due to high intra- and inter-subject variability. It has been proposed that the apparent degradation of RIF. 3-FRSV and INH could possibly undergo Schiff's reaction to form a complex. INH could react with RIF in this manner, which could account for the instability of RIF when present together with INH. This interaction could also occur between RIF and PYZ, however, it has frequently been observed that INH caused further RIF stability reduction compared to PYZ.

Permeability studies have demonstrated that RIF is well absorbed from the stomach due to its solubility, which has been shown to be maximal between pH 1–2. INH, although demonstrating solubility in the gastric environment, is comparatively well absorbed from all three segments of the small intestine. RIF and INH thus exhibit regional specific permeability, and the bioavailability problems associated with RIF could be overcome by developing an FDC in which the delivery of the two drugs is segregated, with RIF released in the stomach and INH in the small intestine. A FDC multiparticulate oral system, which boasts ease of manufacture and directly attacks RIF bioavailability concerns and poor patient compliance with existing FDC anti-TB formulations, is yet to be globally developed.

D.4 Directly Observed Therapy (DOT) for Tuberculosis

There are three groups of people for whom adherence is important: people under evaluation for suspected tuberculosis (to ensure they complete the diagnostic regimen or start treatment); people receiving prophylaxis (preventive therapy), where anti-tuberculosis drugs are given to people exposed to tuberculosis or thought to be at particular risk; and people with diagnosed tuberculosis in whom completion of treatment helps ensure cure. Adherence to a tuberculosis treatment programme requires accessible and appropriate health care. People need to be diagnosed correctly, provided with information about their disease and the need for completion of treatment, and supplied with appropriate outpatient drugs.

But even where these services are available, people may not adhere to the intended regimen. Healthcare providers have responded by developing a variety of specific measures to improve adherence.

These interventions are aimed at influencing the behavior of healthcare personnel, the organization of the service, or the behavior of the person with suspected or confirmed tuberculosis.

DOT seeks to improve the adherence of people to tuberculosis treatment through health workers, family members, or community members directly observing them taking their anti-tuberculosis drugs. This approach was first adopted in Madras, India, and Hong Kong as early as the 1960s and it is now widely recommended for the control of tuberculosis. The advantages of DOT are that people can be closely monitored and that there is a social process with peer pressure that may improve adherence. On the other hand, the disadvantages associated with DOT are that it moves away from adherence models of communication with cooperation between patient and provider back to a traditional medical approach with the patient as the passive recipient of advice and treatment; resource implications for such a policy are substantial, particularly in low income and middle-income countries where the case load is high; and it may make adherence worse if it is rigidly applied in an authoritarian setting or where people are expected to travel considerable distances to have their treatment supervised.

The World Health Organization (WHO) promotes another version of DOT called 'directly observed therapy, short course' (DOTS). This is a comprehensive tuberculosis management programme that focuses on low-income countries. DOTS is a five element strategy for control of tuberculosis consisting of political commitment, improved laboratory analysis, direct patient observation while swallowing each dose of medication, a drug supply that provides for the correct complete short course anti-tuberculosis drug combination for free, and a reporting system that documents the progress in curing the patient (WHO 1997).

The WHO believes that direct observation is a key element for the success of DOTS and has retained it in more recent definitions of the DOTS strategy, although their documentation has clearly taken into account criticisms of their blanket policy.

For example, the WHO states that the third component of their expanded strategic framework is standardized short-course chemotherapy to all cases of TB under proper case-management conditions including direct observation of treatment" (WHO 2002). The recent progress report mentions direct observation for at least" the first two months of treatment (WHO 2004). Even so, how this is interpreted in countries varies, and direct observation by a health worker remains national policy in China.

D.5 DOT Compared with Other Delivery Strategies[85]

DOT versus Self Administration of Treatment

Treatment outcomes were similar among participants in the DOT and self-administered treatment arms. There was no statistically significant difference between the interventions for the number of people cured or completed treatment. The point estimate of the relative risk was close to 1, and no difference was demonstrated with either fixed-effect or random-effects models. The effect size was similar in the two groups.

DOT: At Home versus At Clinic

Cure or treatment completion was similar for those observed by a family member or health worker in a single trial. The cluster randomized trial, which evaluated DOT by a community health worker, found no difference for sputum conversion at two months (OR 0.62, 95% CI 0.23 to 1.71) or cure at the end of treatment (OR 1.58, 95% CI 0.32 to 7.88).

DOT: Family Member versus by a Community Health Worker

There was no statistically significant difference in the number of people cured or who completed treatment (RR=0.97, 95% CI 0.90, 1.05).

DOT for Intravenous Drug Users

There was no statistically significant difference in the number of people who completed twice-weekly clinic-based DOT and those on daily self administration of treatment (RR=1.02, 95% CI 0.89, 1.18).

85. Volmink J, Garner P. Directly observed therapy for treating tuberculosis. *Cochrane Database of Systematic Reviews* **2006**, *Issue 2*. Art. No.: CD003343. DOI: 10.1002/14651858.CD003343.pub2.

For participants receiving prophylaxis, there was no statistically significant difference in the number who completed treatment between those allowed to choose their DOT location and those receiving DOT at a community clinic (RR=0.88, 95% CI 0.63, 1.23).

Given the prevailing support for DOT-based programmes, these findings are important. The benefits associated with DOT programmes in observational studies may be attributable to simultaneous interventions rather than direct observation being the key adherence-promoting strategy.[86] A qualitative study notes that the implementation of DOT is in the process of shifting from being a rigid model involving observation of drug swallowing to one which includes an array of incentives and enablers for supporting the patient.[87] Within such a package of patient-centered interventions it remains to be established whether direct observation is necessary at all.

Of interest in this regard are the findings of a cluster randomized trial in a rural South Africa in which motivation and support from a lay health worker was shown to be more effective in ensuring treatment than a conventional DOT-based service.[88] People with tuberculosis are often poor and encounter numerous barriers to treatment adherence. Strategies aimed at reducing social and health system barriers may therefore be preferable to coercive approaches that impact negatively on patient autonomy.

86. Volmink J, Matchaba P, Garner P. Directly observed therapy and treatment adherence. *Lancet* **2000**; *355 (9212):* 1345-50.

87. Macq JC, Theobald S, Dick J, Dembele M. An exploration of the concept of directly observed treatment (DOT) for tuberculosis patients: from a uniform to a customised approach. *International Journal of Tuberculosis and Lung Disease* **2003**; *7(2):* 103{9.

88. Clarke M, Dick J, Zwarenstein M, Lombard CJ, Diwan VK. Lay health worker intervention with choice of DOT superior to standard TB care for farm dwellers in South Africa: a cluster randomised control trial. *International Journal of Tuberculosis and Lung Disease* **2005**; *9(6):* 673{9.

E. Potential Directions for ICTPH

A focus on RDTs may be of interest considering that rapid and accurate diagnosis of TB is essential for the therapeutic interventions to become more optimal. On the preventive front, ICTPH can explore deployment strategies including supplements or alternates to DOTS. On the curative front, ICTPH can partner with pharmaceuticals active in R&D pertaining to new drug delivery systems for TB. However, it maybe most useful from a public health perspective to focus on the development and deployment of better RDTs. ICTPH can also play a major role in designing studies for HTA assessment including health economics pertaining to TB.

- Improve current test performance characteristics.
- Obtain, in several areas, qualitative and quantitative information that could be used to develop a model for the appropriate introduction or expansion of the use of diagnostic tests (especially RDTs) at the peripheral level, aiming at their optimal deployment.
- Assess the feasibility and acceptability of introducing RDTs in selected situations, such as use in isolated communities and for use by private health providers.
- Assess the role of supplemental or alternate strategies to DOTS
- Health economic studies on RDTs and different deployment strategies for drug delivery like DOTS. It is necessary to conduct a comparative cost effectiveness analysis from a societal perspective. The test needs to be evaluated for its ability to detect different strains including from different geographical locations and resistance to drugs, and the quantum of support infrastructure and training needed. The test needs to be evaluated from a patient and organizational perspective including the impact of the test on several aspects of management of TB.

E.1 Potential Tests of Interest

a. Loop-mediated isothermal amplification (LAMP) technology

b. Nucleic Acid Amplification Tests (NAATs)

c. Mycobacteriophage based tests

The focus should be on testing the accuracy of the test in diverse field and hospital settings. It is also necessary to conduct a comparative cost effectiveness analysis from a societal perspective. The test needs to be evaluated for its ability to detect different strains including drug resistant strains from different geographical locations, and the quantum of support infrastructure and training needed. The test needs to be evaluated from a patient and organizational perspective including the impact of the test on several aspects of management of tuberculosis.

F. Abbreviations

ADA	Adenosine Deaminase
AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
AMTD	Amplified Mycobacterium Tuberculosis Direct
BCG	Bacillus Calmette – Guerin
CFP	Culture Filtrate Protein
DC	Dendritic Cells
DOT	Directly Observed Therapy
DOTS	Directly Observed Therapy Short Course
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme –Linked Immunosorbent Assay
ELISPOT	Enzyme- Linked Immunospot Assay
EMB	Ethambutol
ETB	Ethambutol
EQA	External Quality Assurance
FDC	Fixed Dose Combination
FP	Framework Programme
HBC	High Burden Countries
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
ICTPH	ICICI Centre for Technologies in Public Health
IFN	Interferon
IL	Interlenkin
INH	Isoniazide
IUATLD	International Union against Tuberculosis and Lung Disease
LAMP	Loop mediated isothermal Amplification
LJ	Loweenstein – Jensen
LTBI	Latent Tuberculosis Infections
MDR-TB	Multi-Drug Resistant Tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
MHC	Major Histocompatibility Complex
MTB	Mycobacterium Tuberculosis
NAAT	Nucleic Acid Amplification Tests

NAP	r-nitro-α-acetylamino-β-hydroxy propiophenone
NTM	Non-Tuberculosis Mycobacteria
NTP	National TB control Programmes
PCR	Polymerase Chain Reaction
PhaB	Phage amplified Biological Assay
PLGA	Polylactic – co – glycolic acid
PLG	poly (DL – lactide – co – glycolide)
PPD	Purified Protein Derivative
PRA	PCR Restriction enzyme Analysis
PYZ	Pyrazinamide
PZA	Pyrazinamide
R&D	Research and Development
rBCG	Recombinant Bacillus Calmette – Guerin
RDT	Rapid Diagnostic Test
RMP	Rifampicin
RNA	Ribo- Nucleic Acid
RIF	Rifampicin
SLN	Solid Lipid Nanoparticles
TB	Tuberculosis
TST	Tuberculin Skin Test
TU	Tuberculin Unit
WHO	World Health Organization
XDR-TB	Extensively Drug Resistance Tuberculosis